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AN INVESTIGATION OF THE EFFECT OF ZINC SULPHATE ON PLANTS

BY GWENDA N. DAVIES, M.Sc.
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(With 2 Text-figures)

FOR many years the flora and fauna of Cardiganshire rivers were seriously affected by the proximity of lead mines. These are now disused, and pollution has diminished, but in times of flood there is still contamination due to seepage from the mines. Layers of peat absorb the lead from water passing through them (James *et al.* 1932), and it was suggested (Hooper, 1937) that colonization of the dumps might produce a layer of humus with a similar filtering action. Lead has little direct toxic effect upon certain plants (Hooper & Newton, 1935; Hooper, 1937), and its presence should not prevent colonization. Chemical analyses showed, however, that zinc was more abundant than lead in many mine streams (Hooper & Newton, 1935), zinc sulphate being more soluble than lead sulphate.

TABLE 1

	Lead p.p.m.	Zinc p.p.m.
Water soaking through dumps at Duffryn Castell	0·1	3·0
Red Stream (Cwm Rheidol)	3·0	36·0
Erwtomau	0·1	2·6

The presence of zinc might therefore be an important factor in preventing colonization, and a series of water-culture experiments was carried out to ascertain its effect on plants. The methods used by Hooper were adopted so that the relative toxicity of lead and zinc could be compared. Numerous workers have studied the effect of zinc on plants, some finding a toxic action (Griffith, 1919), others a stimulating one. In all cases zinc stimulated fungal growth.

METHODS USED IN CULTURE EXPERIMENTS

Ten plants of Carter's '50 Days' Dwarf French beans were grown in each of the following concentrations of zinc sulphate: 200, 100, 50, 10, 5 and 1 p.p.m., and were compared with a control series containing no zinc. Graded seeds were germinated in sawdust and then transferred to culture bottles. The solutions were renewed fortnightly and aerated three times a week. The appearance of the plants was noted and typical specimens were photographed. At the end of the experiments the plants were dried and weighed individually, and the average dry weight for each concentration calculated.

Phosphate in the nutrient solution precipitates the zinc as zinc phosphate which is almost insoluble. By using culture solution A (Table 2) where the phosphate was introduced as zinc phosphate, it was possible to keep the zinc sulphate soluble, and it was hoped that sufficient phosphate would be available for plant growth. For comparison, experiments were set up using culture solution R (Brenchley, 1914*b*) (Table 2) which contains much soluble phosphate.

Chemical analyses of culture solutions A and R containing equal amounts of zinc sulphate gave data on this question of zinc precipitation. Ammonium mercuri thyocyanate was found to be a specific reagent for zinc, and micromethods were adopted for the calculations. The results obtained are shown (Table 3).

Much zinc was precipitated in solution R and an increase in zinc occurred in solution A, probably due to the release of some zinc from the zinc phosphate. Chemical analyses showed that phosphate introduced in any form affects the zinc concentration, and this variation must be considered in the results.

TABLE 2

Culture solution A		Culture solution R	
Sodium nitrate	1·0 g.	Potassium nitrate	1·0 g.
Potassium nitrate	0·4 g.	Magnesium sulphate	0·5 g.
Magnesium chloride	0·2 g.	Calcium sulphate	0·5 g.
Calcium nitrate	0·2 g.	Sodium chloride	0·5 g.
Zinc phosphate	0·5 g.	Dipotassium hydrogen phosphate	0·27 g.
Boric acid	0·001 g.	Potassium dihydrogen phosphate	0·30 g.
Manganese sulphate	0·001 g.	Boric acid	0·001 g.
Ferric chloride	Trace	Manganese sulphate	0·001 g.
Distilled water to make up to 1 l.		Ferric chloride	Trace
		Distilled water to make up to 1 l.	

TABLE 3

Amount of zinc introduced as zinc sulphate p.p.m.	Amount of zinc in solution in culture solution A p.p.m.	Amount of zinc in solution in culture solution R p.p.m.
45	80	8
11	33	7
0	16	0

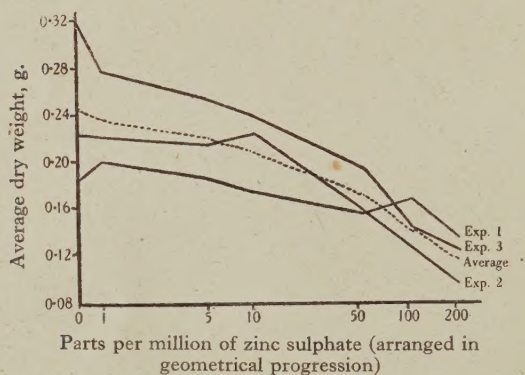


Fig. 1. Average dry weights of plants grown in three experiments using culture solution A.

RESULTS OF CULTURE EXPERIMENTS

Experiments introducing culture solution A

From the appearance and the dry weights of plants in three experiments it was evident that zinc sulphate is definitely toxic. The majority of the plants supplied with more than 100 p.p.m. zinc sulphate were dead after a few weeks, many of them suffering from fungal attack. Root development was poor, probably due to phosphate deficiency. Fig. 1 shows the average dry weights of the plants grown in the various concentrations of zinc sulphate in the three experiments. The general tendency for the dry weights to decrease with increase in the zinc concentration gives a striking indication of the toxicity of zinc sulphate.

Experiments introducing culture solution R

Plants grown in the three experiments attained a far greater size than any grown in solution A. This was due to the phosphate which stimulated growth, and also precipitated much of the zinc. Fig. 2 shows the average dry weights of the plants grown in the several concentrations of zinc sulphate in the three experiments. The dry weights are far greater than those in Fig. 1. Exp. 1 produced smaller plants as it was carried out in the winter months when growth rate was at a minimum. The contours of these graphs are different from those in Fig. 1. The dry weights are variable in low concentrations and a definite decrease does not occur until 50 p.p.m. of zinc sulphate have been introduced. Precipitation took place, and only when 50 p.p.m. of zinc sulphate had been introduced was there sufficient zinc left in solution to exert a toxic action.

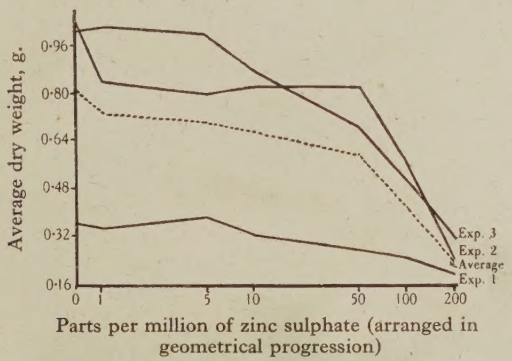


Fig. 2. Average dry weights of plants grown in three experiments using culture solution R.

Summary and conclusions

In spite of the difficulty of keeping the zinc concentration constant, sufficient evidence has been supplied by the experiments to prove that zinc sulphate is definitely toxic to plant growth. Its presence on the mine dumps is undoubtedly one reason for the lack of vegetation. Another reason may be phosphate deficiency, as any phosphate in the soil would combine with the zinc and be unavailable for plant growth. It is suggested that liberal phosphate dressings on the mine dumps would have a beneficial effect. Much zinc would then be precipitated, and, in addition, sufficient phosphate would be left to encourage plant growth.

If, as these experiments suggest, such treatment meets with a certain measure of success, it should be possible to establish bands of vegetation at the base of the mine dumps, and so help to prevent polluted water from reaching the rivers.

This work was carried out under the direction of Prof. Lily Newton, whose interest and advice are gratefully acknowledged. The chemical analyses were carried out for me in the Chemistry Department of this college, and I wish to express my thanks to Prof. T. C. James of Aberystwyth, and Dr Brenchley of Rothamsted for their helpful advice on the chemical aspects of the work.

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INVESTIGATIONS ON THE INVASION OF MUSHROOM BEDS BY *PSEUDOBALSAMIA MICROSPORA*

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(With 1 Text-figure)

The fungus pests with which the grower of cultivated mushrooms (*Psalliota* spp.) is concerned may be placed in either of two groups according to the manner in which they reduce cropping. One group, which includes *Mycogone perniciosa* (bubbles) and *Verticillium Malthousei*, causes damage by parasitizing the mushroom itself. The other group, including such fungi as *Oospora fimicola* (white plaster mould) and *Papulaspora byssina* (brown plaster mould), occupy the bed and act as competitors. In addition, some of these 'weed fungi' may discourage growth of the mushroom by production of toxins, and it is the object of the present paper to discuss one of these, viz. the recently recorded *Pseudobalsamia microspora*, which has been named 'Truffle' owing to its close relation to the Tuberales or Truffle family.

Occurrence of the fungus

The fungus was first described in the U.S.A. by Diehl & Lambert (1930) from material received in the winter of 1929 from a mushroom grower in Ohio. The complaint was made that it was 'filling his beds and completely stopping the production of mushrooms'. Later, the trouble was reported from several other States and Lambert (1930) recorded that one grower alone suffered a loss of at least $100,000. The same author (1932) remarked: 'It is clear that this disease, which was unknown before 1927, is already a menace to the industry and threatens to become a limiting factor.'

The first record of Truffle in this country was made by Williams (1936) who discovered it in the experimental mushroom house at the Cheshunt Experimental and Research Station. Since then, five instances of the occurrence of the fungus have been investigated by the present writers; three in Kent, one in Surrey and one in the Willesden district of London.

The first specimens of fruiting bodies of the fungus were received from a mushroom grower near Maidstone, Kent, on 11 Aug. 1938. The attack had occurred on two adjacent ridge beds in a converted brick-built oast house. The ascocarps formed a dense covering on both sides of a 3 ft. length of one ridge and on both sides of a 1 ft. length of the other. Individual ascocarps were cream in colour with, in some, a rusty red tinge and on removing them from the soil their shape was seen to be subspherical with a wrinkled surface agreeing with Lambert's comparison with the appearance of calves' brains in miniature (Fig. 1). The fungus was also found to a depth of several inches below the casing, the deeper ascocarps being less wrinkled and more nearly spherical. The beds had been spawned in the spring, the first mushrooms had been picked on 16 June, and the invading fungus had not been observed until towards the end of cropping. The grower removed the affected sections of bed and disinfected the site as soon as the trouble was identified. No further spread occurred and loss was small.

The next specimens of Truffle were received on 13 Aug. 1938, from a London (Willesden) grower who enquired whether the fungus bodies were merely deformed mushrooms. The outbreak had occurred in a floor bed which had been cropping since 13 June, and the fungus had been first noticed a few days before the specimens had been sent for identification. The total area of the bed, which was experimental, was 22 sq. ft. Of this, 6 sq. ft. were affected. The ascocarps were similar to those described above. As in the first instance, the affected section of the bed was removed and the site disinfected. The recurrence of Truffle in the house has not been reported. In both the above outbreaks, only small portions of bed were affected.

In the third instance, a serious loss of crop resulted on a large mushroom farm at Farnham, Surrey. On 13 Sept. 1938, the grower reported that some of his beds were infested by an unidentified mycelium which was causing a reduction of crop. Samples of the affected compost were received on 1 Nov., when ascocarps were present and the fungus was recognized as Truffle. A visit was made to the farm two weeks later. The fungus was found to be present in one of eight large houses which were being run on a system of continuous cropping. Ascocarps, which were numerous throughout the casing soil and the compost, were in different stages of disintegration. Some were moist, soft and flattened, being buff yellow in colour, while others were represented by small ochraceous masses of powder. It was evident that the stage had been reached at which the mature spores were being released by decay of the surrounding fleshy tissue. The colour and texture of the compost in the beds indicated that it had been well 'run' with spawn, but very little mycelium was visible. A handful of the compost drawn from the bed had a smell reminiscent of that of rotting lawn mowings together with a trace of chlorine. The house contained twenty-two ridge beds, each 120 ft. in length, and all of these were affected.

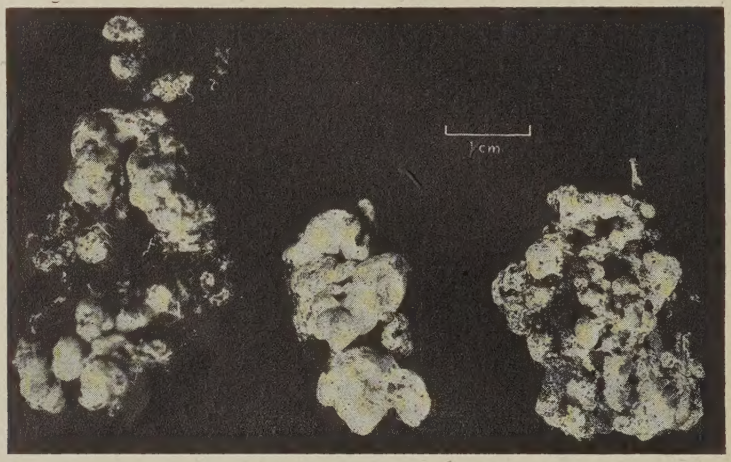


Fig. 1. Ascocarps of *Pseudobalsamia microspora* removed from surface of casing soil. Maidstone, Kent, Aug. 1938.

The beds had been spawned at the beginning of August and casing had been done one week later. The grower stated that the crop had been poor from the start and he estimated that the yield had been reduced by at least two-thirds. He later reported that although Truffle had developed in outdoor beds in the summer of 1939, the fungus had not again been seen in any of the indoor beds.

In the first two occurrences reported from commercial houses, the invading fungus was not noticed until the beds had been in production for nearly two months. In the third, the fungus was not sent in for identification until three months after spawning. This delay is in agreement with the observations of Diehl & Lambert (1930) who stated that during the early stages of the crop, the yield of mushrooms in infested beds is usually normal. Under certain conditions, however, it is evident that the Truffle materially reduces the yield during the latter half of the cropping period.

The two remaining occurrences of Truffle were brought to notice during laboratory experiments on casing soils. Amongst various soils two had been collected from two different places in west Kent and were being tested for physical and chemical properties in relation to mushroom growing. After a few weeks in the laboratory in contact with compost which had been fully 'run' with spawn, numerous ascocarps of Truffle developed in both compost and soil.

Origin of invasion

It is of great practical importance to know by what means the Truffle fungus is introduced into a mushroom house in the first instance. In his preliminary account Lambert (1930)

remarked: 'Circumstantial evidence suggests that it is a widely distributed soil organism which is inconspicuous until it enters the mushroom house and finds a favourable medium for extensive development in the bed.' Referring to its means of introduction into the house, Diehl & Lambert (1930) considered it probable that it enters with the compost. In a later paper, Lambert (1932) concluded that the source of the fungus was not then known because it had never been found outside mushroom houses. In his view there were two types of infestation that must be considered: (1) the initial infestation, which is often confined to a few small areas on the bed, and (2) the re-infestation, which frequently involves an entire house or several houses.

The attack recorded by Williams (1936) was discovered in a bed which was being used for the comparison of different types of casing soil. It was at first confined to soil from one particular source, but it later spread throughout the bed, almost completely preventing the development of the mushrooms. From his observation of the course of the attack, Williams concluded that the casing soil was the source of the infection.

The outbreaks near Maidstone and in London were in each case localized to one small area of the bed and the attack did not appear elsewhere in the house after the infested parts were removed. Had the fungus been present in the horse manure at any stage prior to the completion of composting, its distribution throughout the heap would have been assured during the turning process and outbreaks would have occurred simultaneously in several parts of the bed.

At Farnham, Truffle was distributed throughout the beds of one house in which soil from a local source had been used for casing. Beds in other houses which were free from the trouble had been cased with imported soil and the fungus did not develop. The local soil had been dug from the surface layers of a nearby field and had been made up into heaps after having been passed through a screen to remove large stones. At the time of their visit the writers collected samples of this soil for examination in the laboratory. Truffle was found to be present. Since the fungus had not previously been observed in any of the houses, re-infestation from an earlier attack was unlikely and evidence again pointed to the soil as the source. The infestation which later occurred in the outdoor beds on the same farm was not investigated by the writers, but since some of the soil on the premises was known to be infected with Truffle, an attack is not surprising.

In the instances referred to above in which Truffle developed from two soils with which experimental work was being carried out, the bulk samples were tested and the fungus was found to be abundant in each. Samples of soil from several other sources were being used in the same series of experiments, but these proved to be free.

Thus, Truffle has three times been isolated directly from the soil and on two occasions circumstantial evidence has strongly indicated that the fungus is introduced with the casing soil.

Detection of the fungus in soils and media used

A simple method of testing soil for the presence of Truffle has been devised. Fresh pure-culture spawn of the white variety[1] of cultivated mushrooms is slightly moistened and is packed into the lower half of a glass boiling-tube. This is then topped with a layer of

[1] According to Lambert (1932), beds of the white variety are as a rule more severely infested than are those of the brown variety.

about ½ in. of the previously moistened soil which it is desired to test. After being plugged with cotton wool, the tube is placed in an incubator kept at a temperature of 83° F., the temperature reported by Beach (1937) as optimum. Where the fungus is present in the soil, a characteristic cottony growth of cream-coloured mycelium fills the matrix of the spawn and the soil itself after a period of about two weeks. A few days later, ascocarps develop freely on the surface of the soil and throughout the contents of the tube. With those soils tested it has been found that other fungi seldom develop sufficiently to inhibit the growth of Truffle and six test-tubes filled in the manner described are enough for each sample of soil.

This test for detecting the presence of Truffle in soil should be helpful to advisory mycologists and others in tracing the source of an outbreak and in finding out whether soil which it is intended to use for casing is likely to be free from the pest. The value of such a test is, of course, limited by the efficiency of sampling.

In further study of the fungus itself, tube cultures have been made from tissue taken from immature ascocarps. The media used were potato-dextrose agar, corn meal agar and Dox agar. The mycelial growth on the first two media was close and fine, but on Dox agar it was fluffy and aerial and in one or two cultures on this medium numerous small ascocarps developed.

Diehl & Lambert (1930) found that of the various media used by them, ascocarps developed best on boiled oats with soil extract. The present writers also found this successful using the method given by Giltner (1921) for the preparation of soil extract. Abundant ascocarps in pure culture were also readily obtained in Petri dishes on pure-culture mushroom spawn which had previously been sterilized by steam. When kept at optimum temperature, ascocarps were formed within ten days from inoculation.

Persistence in mushroom houses

The time since Truffle has been recognized in this country has been too short to know whether it is likely to be a constantly recurring pest or whether its appearance is spasmodic. Lambert (1932) from his experience in the U.S.A. wrote that 'once Truffle disease has appeared in a mushroom house, re-infestation usually occurs and frequently the disease becomes increasingly prevalent in subsequent crops. It seems improbable that casing soil has been the source of severe re-infestations because in many instances the fruiting bodies of the Truffle fungus developed before the casing soil was applied. Circumstantial evidence indicates that the fungus follows one or more of three courses: It lives over from one crop to another inside the mushroom house; it persists in the soil of the composting grounds; or it develops in soil which is later added to the compost. If it remains in the mushroom house, the fact that it persists in spite of ordinary sulphur or formaldehyde fumigation indicates that the spores can withstand this treatment or that the fungus escapes the fumigant by penetrating deeply in the bed boards. The fact that the disease frequently reappears in subsequent crops in the same spot in the bed points strongly to the theory of fungus penetrating into the bed boards. Spores are probably widely distributed at the time of emptying the beds and many of them undoubtedly are blown on to the composting ground where it is possible that they may remain to re-infest a new compost heap. In test-tubes these spores have been shown to be capable of withstanding temperatures of at least 180° F. for 5 hr.

Therefore they may easily live through the temperatures generated in the compost heap and in the house during the final fermentation of the bed.'

In none of the cases investigated by the writers has re-infestation occurred and, in two of the three commercial houses, this may have been due to the sections of the affected beds having been removed probably before the ascocarps reached the stage of producing mature spores. As all of these beds were floor beds, the possibility of the fungus penetrating woodwork was eliminated but since the natural habitat of the fungus appears to be the soil, penetration into the hard earth floors of the houses might be expected. In the instances under review, the floors were liberally treated with fungicides after the beds had been removed.

Prevention and control

From the above observations it would appear that prevention consists of avoiding the use of infected soils for casing. Complete control in some instances, as described on p. 85 can be effected if steps are taken early enough to remove infected portions of bed. Where the fungus has become established throughout the beds it is already too late to consider control methods, but rather prevention of re-infestation. The following points are pertinent. Survival of spores at high temperatures much reduces the likelihood of the elimination of the fungus by the temperatures reached during composting or during the peak heat period normally reached in mushroom houses prior to spawning. Furthermore, it is doubtful whether the steam sterilization of casing soil is usually efficient enough to raise all of the soil beyond the thermal death point for a sufficient length of time. It seems probable, therefore, that once spores have been distributed about mushroom growing premises, the fungus will reappear in the beds if growing conditions are suitable. As already mentioned, Lambert (1932) found that spores of Truffle in test-tubes are capable of withstanding temperatures of at least 180° F. for 5 hr. The writers tested this point with spores produced in cultures made in mushroom spawn and although they did not succeed in inducing germination after 5 hr. at 180° F., germination occurred readily after 4 hr. at this temperature.

Most mushroom growers now clean and disinfect their houses between crops, either by spraying the interior, or by fumigation, or by both methods. Spraying with formaldehyde solution and fumigation with sulphur dioxide are methods commonly used. Beach (1937) remarked that there were indications that Truffle survived in bed boards in spite of this treatment. Bewley & Harnett (1938) reported that all attempts to destroy the infection in experimental beds at Cheshunt in 1936 failed and they added that the fungus was to be regarded as a distinct menace to mushroom work.

In spite of the resistance of Truffle to various attempts at disinfection, it appears to be sensitive to growing conditions. Thus, according to Beach (1937), mushroom spawn will grow in alkaline conditions which are not tolerated by Truffle; the latter is doubtless favoured by the acidifying effects of spawn growth, and it therefore develops after spawn has run. The usual very alkaline reaction of fresh manure does not permit the growth of Truffle.

With an optimum temperature for growth of 83° F., the occurrence of Truffle is associated largely with warm weather and if the presence of the fungus is suspected, it is advisable to grow the mushroom crop at a temperature as low as is consistent with good practice. According to Beach (1937) Truffle will not grow below 52° F. In common commercial

practice, temperatures suitable for the spread of Truffle mycelium would obtain in mushroom houses during the 'running' period of the mushroom spawn. When the first mushrooms appear and the temperature of the house is reduced to a point near the minimum for the growth of the Truffle fungus, development of ascocarps on the surface of the beds would be unlikely and there would consequently be no visible sign of its presence. It is noteworthy that in the three occurrences of Truffle in beds in commercial houses, ascocarps were not seen before cropping was well advanced and that the trouble was noticed only in the summer months. Beach (1937) pointed out that only a few occurrences of Truffle were reported by mushroom growers in Pennsylvania from 1934 to 1937, and that while growers who had had the fungus in their houses had endeavoured to disinfect properly and remove all infected waste, the negative results in various experiments indicated that the organism often disappeared through natural causes.

The discovery of Truffle by the present writers in soils taken from three different localities and the strong circumstantial evidence of its occurrence in two others suggests that it is an organism widely distributed in nature. If this is so, it is remarkable that it has not been earlier recognized as an invader of mushroom beds. Lambert (1932) has, however, pointed out that the ascocarps are often mistaken by growers in the United States for deformed or rudimentary mushrooms and it is probable that growers in this country have been similarly misled. It seems also to the present writers that the mycelium of the fungus might develop in a bed, but that conditions might not be favourable for ascocarp development on the surface of the casing soil; thus there would be no evidence of its presence for the grower to see. Such infestations could well account for some of the numerous unexplained crop failures of the past.

Summary

1. Five occurrences in the south-east of England of *Pseudobalsamia microspora*, an invader of commercial mushroom beds, are described.

2. From examination of the evidence it is concluded that, in the first instance, the fungus is introduced into the mushroom beds with the casing soil. Isolation of the fungus from freshly dug soils supports this view and indicates that it is a common soil organism.

3. An easy means of detecting the presence of the fungus in soils is described and cultural methods are discussed.

4. The most practicable method of preventing the introduction of the fungus into beds is to avoid using contaminated soil. It is shown that in some instances early attacks can be controlled by the removal of the affected part of the mushroom beds and treatment of the site with disinfectants. Factors influencing re-infestation of beds are discussed.

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A WILT DISEASE OF GODETIAS AND OTHER ORNAMENTAL PLANTS

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(With Plate 5)

In 1936 Prof. F. T. Brooks observed a wilt of godetias in his garden, which was associated with attack on the stems by a pycnidial fungus. The spores were hyaline, cylindrical, and unicellular or uniseptate. The fungus was isolated and experiments showed that it was pathogenic to godetias. Miss E. M. Halley began an investigation of the disease, but was unable to continue the work, and the material was placed in my hands, together with a similar disease of sweet sultan (*Centaurea moschata*). Subsequently, clarkias and antirrhinums were found to be infected in various gardens by the same or a closely allied fungus. The fungi isolated from these four hosts were tested for pathogenicity on a wide range of herbaceous ornamental plants grown in the open and in a glasshouse.

Symptoms of the disease

The typical symptoms of naturally infected plants of antirrhinum, clarkia, godetia and *C. moschata* are the browning (blackening in *C. moschata*) of the stem, mainly in the lower portions of the plant, the extension of the lesion and final encirclement of the stem. The plants subsequently wilt. Infection is commonly observed to centre round the nodes near soil-level, and occasionally parts of the plant at some distance from the ground are also affected. Abundant pycnidia develop later upon the dead shoots.

Typical symptoms following the wound inoculation of stems of godetia and clarkia with fungus isolations designated as G, GX, Cl, SS and AI, take the form of a slight browning of the stem at the point of infection. This brown area spreads as infection proceeds until the lesion encircles and extends for some distance up and down the stem, which becomes very brittle. The upper parts of such plants appear healthy under these conditions for a considerable time, although leaves in the vicinity of the lesions wilt and fall. Plants of *C. moschata* inoculated with these fungi show a blackening of the stem rather than a browning, but the progress of the disease is essentially similar. The affected shoots eventually wilt completely and die; this is followed by the formation of pycnidia on the parts of the shoot affected.

Origin of the cultures

Notes upon the fungi isolated from wilted plants of godetia, clarkia, *Centaurea moschata* and antirrhinum are given as follows:

G. Isolated from pycnidia by Prof. Brooks in August 1936 from godetia and received by the writer in October 1938. The fungus had been inoculated into and re-isolated from godetia, var. Sybil Sherwood, in May 1937.

GX. Isolated from pycnidia by the writer on 24 July 1939 from a wilting plant of godetia, var. Sybil Sherwood, at the Cambridge Botanic Garden. Further isolations made at a later date did not differ from this.

Cl. Isolated from pycnidia by the writer on 24 July 1939 from a wilting plant of *Clarkia elegans*, var. Ruby King, at the Cambridge Botanic Garden. Later in the season, each of several colonies of

clarkia in the herbaceous border wilted and died. Pycnidia formed freely upon the affected parts of the stems. The same disease was also observed at the Botany School Field Station in August 1939, and the causal fungus appeared to be identical in culture with the isolate obtained in July; these isolates were subsequently shown to be pathogenic to *Clarkia elegans*.

SS. This isolate was derived by Prof. Brooks from pycnidia on wilted plants of *Centaurea moschata* in a Cambridge garden in July 1938. The writer also observed the disease on *Centaurea moschata* in the Botanic Garden in July 1939; there were abundant pycnidia on the lower parts of the stems and the fungus isolated was found to be pathogenic and was identical with that obtained in 1938.

AI. Isolated by the writer from wilting shoots of otherwise vigorously growing plants of antirrhinum at the Cambridge Botanic Garden in June 1939. The fungus was found to be pathogenic to antirrhinum. The same disease was seen upon antirrhinums on other occasions.

Host range

The pathogenicity of the pycnidial fungi under investigation was tested over a wide range of hosts (Tables 1 and 2). The fungi were in each case introduced as mycelium through stem-wounds, the points of inoculation were then wrapped with tinfoil, and the plants placed under bell-jars for 48 hr.; under these conditions, each was found to be pathogenic to its respective host.

Table 1. Godetia, clarkia, *Centaurea moschata*, antirrhinum

	G	GX	Cl	SS	AI
Godetia:					
var. Schamini	3	3	3	3	3
Cherry Red	3	3	3	3	3
Sybil Sherwood	3	3	3	3	3
Azaleiflora plena	3	3	3	3	3
Kelvedon Glory	3	3	3	3	3
Lavender Gem	2	2	2	2	2
Wild Rose	3	*	*	2	3
Clarkia elegans:					
var. Scarlet Queen	3	3	3	3	3
Centaurea moschata (White)	3	3	3	3	3
Antirrhinum:					
var. Amber Queen	o	3	o	3	3
Malmaison	o	3	o	3	3
Rose Queen	o	3	o	3	3
The Rose	o	3	o	3	3

3, markedly pathogenic. 2, weakly pathogenic. o, not pathogenic. *, no test.

Tables 1 and 2 show that, if the results of inoculation of plants other than antirrhinum, scabious and *Centaurea Cyanus* are considered, the behaviour of the fungi on different hosts is essentially the same. In the antirrhinum inoculations there is a marked difference between GX, SS and AI which are strongly pathogenic, and G and Cl which appear unable to attack this host. With scabious, only GX and Cl become established, and although strong lesions were produced, the plants were not seriously affected for a considerable time after inoculation; of the isolates G, SS and AI with which *C. Cyanus* was inoculated, only SS was effective. Different varieties of each host plant have also been inoculated and slight variation has been observed in the degree of susceptibility of individual varieties. With godetia, varieties Cherry Red and Schamini are slightly less susceptible to G and SS than the other varieties tested with the exception of Lavender Gem, which is comparatively resistant to all the pycnidial isolates.

Table 1 is largely compiled from a comprehensive experiment carried out in April 1940 in which all the plants were inoculated as nearly as possible at the same time and under the

same conditions. Six plants of each kind were inoculated with the isolates G, GX, Cl, SS and AI respectively, and adequate controls were kept. The results accord closely with inoculations carried out previously except for the infection of antirrhinum by GX and SS. Further inoculation experiments carried out with antirrhinum have confirmed the pathogenicity of GX and SS to this host.

TABLE 2. *Other ornamental plants*

	G	SS	AI	GX	Cl
Scabious	0	0	0	2	2
Centaurea Cyanus	1	2	1	—	—
Ageratum	0	0	0	—	—
Calliopsis	0	1	0	—	—
Callistephus	1	1	1	—	—
Cineraria	1	1	1	—	—
Dahlia	1	1	1	—	—
Helianthus annuus	0	1	1	—	—
Helichrysum	0	1	0	—	—
Matricaria	0	1	1	—	—
Zinnia	0	1	*	—	—
Matthiola (Garden Stock)	1	1	1	—	—
Cheiranthus Cheiri	1	1	*	—	—
Calceolaria	1	0	0	—	—
Nemesia	0	1	0	—	—
Alonsoa	*	0	0	—	—
Mimulus	0	0	0	—	—
Petunia	0	1	0	—	—
Schizanthus	0	0	0	—	—
Gypsophila elegans	0	0	0	—	—
Phlox Drummondii	0	0	0	—	—
Delphinium (Larkspur)	0	0	0	—	—
Eschscholtzia Californica	*	0	0	—	—
Oenothera biennis	*	3	*	—	—

3, markedly pathogenic. 2, weakly pathogenic. 1, very weakly pathogenic. 0, not pathogenic. *, no test.

TABLE 3

	P. Antirrhini	*P. Richardiae*	*D. Lycopersicum*
Godetia:			
var. Sybil Sherwood	—	—	+
Schamini	—	+	+
Wild Rose	+	+	+
Clarkia elegans:			
var. Glorious	—	—	—
Antirrhinum (Red)	+	—	—
Tobacco	*	—	*
Gypsophila elegans	*	+	*

+, pathogenic. —, non-pathogenic. *, no test.

In experiments made by Miss Halley the godetia fungus (G) failed to infect plants of tobacco (White Burley), tomato (var. Kondine Red), *Epilobium hirsutum*, *E. angustifolium* and *Oenothera biennis*, and was only weakly pathogenic to *Clarkia elegans*. In one experiment, of four plants of a red variety of antirrhinum inoculated with the godetia fungus (G) through stem-wounds, one became infected. Experiments made by the writer have shown *Oenothera biennis* to be susceptible to SS.

Table 3 is a summary of the results obtained by Miss Halley in inoculation experiments in which cultures of *Phyllosticta Antirrhini* Sydow, *P. Richardiae* Brooks and *Didymella Lycopersicum* Klebahn were used upon a number of hosts for comparison with the godetia fungus (G).

Mode of infection

In most of the preliminary experiments for the purpose of investigating the host range and for the maintenance of the pathogenicity of the fungi by passing them through their respective hosts, inoculations were made by introducing mycelium of the fungus into stem-wounds. A description of typical symptoms following such inoculations is given on p. 91. Later, however, a variety of methods was used in order to try to ascertain the means of entry of the fungus under natural conditions.

Infection through the leaf-axils

Plants of godetia, clarkia and antirrhinum were inoculated by placing drops of spore suspensions of the fungi in the leaf-axils. The plants were put under bell-jars for 60 hr. In one-half the number of plants the axils were undamaged, and in the remainder the axillary shoots were removed. The plants from which the axillary shoots had been removed were readily infected with their respective fungi and godetia and clarkia also by the other fungi, whilst infection only occasionally occurred where the spore suspension had been placed in the uninjured axils. These exceptions may have been due to insect damage which had escaped observation.

Infection through cut stem extremities

Infection was also initiated by placing drops of spore suspension on cut terminal shoots; the fungus grew down the infected shoot, developing mainly in the pith and cortex.

Leaf-inoculation of plants in pots

Plants of godetia, var. Schamini were inoculated with a spore suspension of G. Six plants were used, two of which were used as controls in which drops of water replaced the spore suspension for inoculation. Drops of spore suspension were placed with a small pipette, on the leaves of two of the plants, the leaves being simultaneously perforated at the point of inoculation with the tip of the pipette: thirty inoculations were made. On two other plants, thirty inoculations were made without damaging the leaves. Of two control plants, the leaves of one were wounded on applying the water-drops, and the leaves of the other were not wounded. The plants were placed under bell-jars for 48 hr.

After 2 days twenty-five lesions developed following the thirty inoculations made on the plants in which mechanical injury accompanied spore inoculation. All the other plants remained unaffected. The lesions were mostly circular and about 3 mm. across. After 5 days, the older parts of the lesions were brown, dry and crisp with a tendency to drop out, producing a 'shot-hole' effect; pycnidia developed in many lesions. The extension of infection was slow and tended to produce a pale green, semi-transparent zone. The rate of spread seemed to depend upon the humidity. The experiment was repeated several times with similar results.

Leaves of *Clarkia elegans* inoculated with drops of a spore suspension of G accompanied by wounding, also developed leaf-lesions. In one experiment where the plants had been inoculated at points of injury but not put under bell-jars, the lesions were so restricted as to be indefinite, but these developed strongly when the plants were put under bell-jars for 48 hr. Infection had apparently taken place but the conditions were not sufficiently humid for rapid spread. Similar results were obtained with antirrhinum, vars. Rose Queen and Malmaison, when the leaves were inoculated with a spore suspension of A1; lesions 2–3 mm. across developed within 4 days.

Leaf-inoculation experiments in the laboratory

The following experiments were made to determine the conditions influencing the germination of the spores on the host. At first, attention was confined to investigating the penetration of godetia by the germ-tubes of isolate G. Infected material was fixed and cleared in lacto-phenol containing a little cotton-blue (see Pady, 1935). Preliminary experiments were made using axillary shoots of godetia, var. Sybil Sherwood, which had been dipped into a dense spore suspension of G and then placed on damp filter-paper in Petri dishes. At intervals of several hours, leaves were removed, stained and cleared.

Influence of the water-film on spore germination. The germination of the spores was at first irregular, apparently due to the uneven distribution of the water-film on the leaf surface. Some of the spores

germinated in about 17 hr. but only those which were on portions of the leaves which had been wet. Others failed to germinate within 42 hr. From these observations it would appear that germination takes place only in the presence of water in the liquid form and does not occur in the absence of a water-film, even in a saturated atmosphere. The cut ends of the shoots which had also been dipped in the spore suspension were readily infected. The experiment was repeated several times using detached leaves and taking care to wet thoroughly the surface to which the spore suspension was applied; separate series of leaves were treated, on upper and lower surfaces respectively and controls were set up in each. Old and young leaves were treated similarly. Germination, although still not uniform, took place in 24–40 hr.

No infection was observed in the above experiments. Many of the germ-tubes grew to considerable lengths, proceeding in directions independent of stomata or epidermal cell junctions. Some grew away from the stomata and some crossed over them: there was no predisposition to enter via the stomata nor was there any evidence that penetration occurs through the stomata. The fungus also appeared to be unable to enter the host through the cuticle directly.

Effect of wounding. An experiment similar to those already made in Petri dishes was set up in duplicate, and in one series the leaves were wounded by pricking them with a glass needle. Each leaf was punctured in about fifty places. The most rapid and vigorous germination of the spores occurred in the vicinity of wounds and the germ tubes tended to grow towards such points of injury. Other spores remote from the wounds did not germinate. Spores upon unwounded leaves germinated poorly and only after a considerable delay. The mycelium resulting from the spores germinating in and near wounds penetrated into the surrounding tissues at the points of damage; a slightly discoloured and disorganized zone soon developed, round which was a further ring of tissues which stained more deeply with cotton-blue. Although only small areas of infection were evident, one considerable lesion developed. The conditions did not appear to be entirely satisfactory for the development of extensive lesions. The influence of wounds in stimulating spore germination, and the growing towards the wounds of the germ-tubes suggested that the sap diffusing into the water-film on the leaf-surface from the injured tissue may play an important rôle in the penetration of the host.

Effect of sap on spore germination. Hanging-drop cultures were set up using spores of G. The spore suspension in distilled water was obtained from a recently re-isolated culture of G growing on Dox's agar. Leaves and stems of godetia, var. Sybil Sherwood, were crushed, and the sap was added in graduated amounts to the spore suspension. The series consisted of drops of suspension with sap added at the rate of 1, 2, 4, 8 and 16 'loops' to 0·5 c.c. A 'loop' consisted of the amount held by a wire loop with a diameter of approximately 3 mm. Controls were set up in which no sap was present and also in which the spore suspension was in sap alone.

From 20 to 80% of the spores germinated within 17 hr. in preparations containing sap, the greatest germination occurring in the higher concentrations. No germination occurred during that time in the preparations to which no sap had been added. After 23 hr. the spores in the preparations without sap were beginning to show distension, but it was not until 43 hr. had elapsed that slight germination occurred; by this time the other preparations had become felted mycelial growths. Similar results were obtained in other experiments, and the same stimulating effect occurred when spores of AI germinated in association with antirrhinum sap. The results of these experiments correspond closely with those of the leaf-inoculation experiments in the laboratory and in the greenhouse.

From the foregoing account it seems likely that, under natural conditions, in the event of insect or other injury (e.g. soil abrasion) to the leaves or stem followed by suitable temperature-moisture relations, germinating spores of the fungus penetrate the host and establish infection.

Spore-spraying experiments

Godetia plants were inoculated by spraying them from an atomizer with a heavy spore suspension of G, the leaves of some of the plants being punctured before the application of the spore suspension: the plants were then placed under bell-jars for 60 hr. No infection occurred in the uninjured plants but small lesions developed slowly at the points of injury in the other plants. A high humidity favoured the subsequent development of these lesions. In two experiments conducted by Miss Halley a total of seventeen unwounded plants were sprayed with a spore suspension in distilled water. In several plants a brown discoloration of the stem was noticeable extending upwards for 1–2 in. from soil-level. Many of the leaves developed necrotic areas. The godetia fungus was isolated from the lesions on the inoculated plants, and *Botrytis* sp. from one control which became similarly affected. None of the plants died during the 4 weeks succeeding inoculation. There is some discrepancy between the results

of these experiments carried out by Miss Halley and myself. Conditions may have been more favourable for infection when she carried out the inoculations.

Soil-inoculation experiments

A number of experiments in which healthy, unwounded godetia plants were transferred to soil inoculated with G did not give positive evidence of infection through the soil. On one occasion, however, G was re-isolated in association with a *Pythium* sp. from a wilting plant. The soil, both sterilized and unsterilized, was inoculated with the fungus growing on various media including beech leaf-mould, wheat grains and kibbled maize. One godetia plant became infected among eight which Miss Halley inoculated through root-wounds with the godetia fungus. It seems probable that infection most commonly occurs through wounds in the stem near ground-level from spores splashed from the soil by heavy rain. It is possible also that the fungus may enter the roots in conjunction with another parasite.

Cultural comparison of the fungi isolated

Dox's agar, on which the fungi grow well, was used as a stock culture medium. The typical growth of GX, Cl, SS and AI isolates was an irregular greyish mat of mycelium, whilst that of G was somewhat paler with less aerial mycelium and a greater abundance of pycnidia, rather paler in colour. The writer understands, however, that the G isolate originally closely resembled the other isolates. The five isolates were compared in culture on Dox's, potato-dextrose, oatmeal and malt agars. The differences between the isolates were most marked on Dox; on the other media the differences were slight. Growths on potato-dextrose and malt agars were vigorous and tended to become heavily pigmented, the cultures becoming almost black, with grey aerial mycelium. On oatmeal agar the growths were somewhat paler and formed a thick mycelial felt.

Certain changes have occurred since isolation. Coupled with a considerable loss in pathogenicity, cultures of both SS and AI became progressively paler in colour and at one stage were almost pure white, the mycelium forming an even mat with an almost total absence of pycnidia. This appearance exactly resembled that of another series of stock cultures of G. The pathogenicity and the typical cultural characters of AI were slowly restored after several passages through the host. A pathogenic strain of SS was again obtained by re-isolating the fungus from dried specimens of *Centaurea moschata* which had been inoculated with SS. Re-isolations were generally made from spores.

The effect upon the cultural characters induced by a change in nutrient conditions may be illustrated by the following observations on G. After re-isolating G from godetia, var. Schamini on malt agar on 4 July 1939, it was sub-cultured on a malt agar slope on which it remained until 27 July. The resulting growth was vigorous and dark in colour. On transference back to Dox's agar, the original cultural characters appeared to have been lost, the growth being flat, with heavy brown pigmentation and an almost slimy appearance. After growing on Dox during five successive transferences, however, the cultures resumed the appearance of the original culture as received by the author. The GX isolate is gradually acquiring a greater resemblance to G. The isolates were formerly almost identical, but subsequent slight diversions of character have occurred and the cultural type is not very stable. Only by frequent re-isolation from the appropriate host has it been possible to retain relative constancy in the cultural characters and pathogenicity of the fungi. The following comparison was made with a view to determining whether the host influenced in any marked degree the cultural characteristics of these fungi. From the comprehensive inoculation experiment made in April 1940 (see p. 92) the fungi were re-isolated on 15 July 1940 from pycnospores on the various hosts. Four days later tube cultures (Dox's agar) of all these re-isolations were made. The five series of cultures (G, Cl, GX, SS and AI) were photographed when 20 days old (Pl. 5). The key to the culture numbers is given in Table 4.

G (1–8). Aerial mycelium very sparse with faint yellowish tinge during the earlier stages of growth, later greyish white, very thin in the upper parts of the tubes. Numerous cream-buff pycnidia. Yellowish crust composed mainly of pycnidia at the bottom of the slopes. The crust appears black at the edges. The cultural characters of G have changed considerably since it was first isolated. All the isolates in the present experiment are essentially the same.

Cl (9–16). Aerial mycelium fairly abundant, greyish mixed with white. Yellowish black crust at the bottom of the slopes as in the G cultures (1–8). Pycnidia sparse. The isolation from *Clarkia elegans* (15) failed to develop. The cultural characters of Cl have changed somewhat since first isolation.

GX (17–28). Aerial mycelium abundant, floccular, greyish white. Pycnidia fairly abundant on the surface of the medium. Black aggregates of hyphae also producing pycnidia at the sides of the cultures.

These cultures are more or less similar in appearance to those of G and Cl when they were isolated originally. With the exception of the culture isolated from godetia, var. Cherry Red (17), the characters of each of the cultures of GX (17–28) are essentially the same. The exception (17) resembles an extreme form of the present cultures of G; aerial mycelium is absent, a great abundance of cream-buff pycnidia is present and masses of cream spores have exuded from them on to the surface of the culture. In the upper part of the tube the appearance is identical with the corresponding part of the G cultures.

SS (29–40). Much aerial mycelium present, whitish at first, turning much darker. Later the aerial mycelium collapsed on the surface of the medium. In this condition the mycelium appears almost black. A black crust of aggregated hyphae and pycnidia appears at the bottom of the cultures and along the sides. One or two of the cultures with greyish white aerial mycelium closely resemble the appearance of the fungus when originally isolated. The cultures are, however, essentially similar to one another.

TABLE 4

	G	Cl	GX	SS	AI
Godetia:					
var. Cherry Red	1	9	17	30	41
Schamini	2	10	18	29	42
Sybil Sherwood	3	11	19	31	43
Azaleiflora plena	4	12	20	32	44
Kelvedon Glory	5	13	21	33	45
Lavender Gem	6	14	22	34	46
Clarkia elegans:					
var. Scarlet Queen	7	15	23	35	47
Centaurea moschata (White)	8	16	24	36	48
Antirrhinum:					
var. Rose Queen	*	*	25	37	49
The Rose	*	*	26	38	50
Amber Queen	*	*	27	39	51
Malmaison	*	*	28	40	52

*, no test.

AI (41–52). Whitish grey felt of aerial mycelium, darker at the sides of the cultures and on the surface of the medium beneath the mycelial felt. Black aggregates of mycelium appear at the bottom of the slopes. Some cultures are a little more grey than others. With the exception of (43), these cultures also are essentially similar but their characters have changed somewhat since the original isolate was obtained. The isolate of AI from godetia, var. Sybil Sherwood (43) is to some extent different in that it resembles the present cultures of Cl more closely than the rest.

The five series of cultures G, Cl, GX, SS and AI form a graduated series of cultural types in the order in which they are listed. Although AI and G seem so dissimilar they are closely linked by the intermediate cultural types of Cl, GX and SS. Passage through a variety of hosts does not appear to influence to any noticeable degree the cultural characters of these fungi.

The perfect stage of the above fungi has not been observed either in culture or on overwintered plants killed by their respective fungi. An ascomycete (*Physalospora* sp.?) which was isolated from many dying godetias and clarkias at the Field Station, and was subsequently shown to be pathogenic, was culturally distinct from the pycnidial fungi investigated.

IDENTIFICATION

All five isolates G, GX, Cl, SS and AI are pycnidial fungi (order Sphaeropsidales) which have been found under natural conditions causing a stem-rot and wilt of their respective hosts. In each of the fungi, the spores are hyaline, cylindrical with rounded ends and approximately 6·0–9·0 × 2·5–3·5μ, 5–40% being uni-septate and the rest aseptate. Occasional bi-septate spores are seen. The number of septate spores present should preclude the fungi from the genera *Phoma* and *Phyllosticta*. The characters of the pycnidia and spores would, however, place them in either the genus *Diplodina* or *Ascochyta*.

Various methods of differentiating these two genera have been accepted by systematists. Bender (1934) relies upon the maculicole habit or otherwise of the pycnidia for separating these genera; those of *Ascochyta* being maculicole and those of *Diplodina* not. The separation of *Diplodina* and *Ascochyta* is regarded by Sprague (1929) and others as being of 'doubtful value'. Grove (1935) separates them according to the relative thickness of the pycnidial wall. Allescher (1899), however, segregates them by regarding the stem-fungi as being in the genus *Diplodina* and leaf-fungi in the genus *Ascochyta*. According to each of these methods of differentiation, the fungi investigated would belong to the genus *Diplodina*.

Identity of AI

In view of the great similarity of the fungi isolated from various hosts in this investigation and of the possibility that they belong to a single species, it was decided first to compare the pycnidial isolate from antirrhinum with the pycnidial pathogenic fungi known to occur on this host, viz. *Diplodina Passerinii* Allesch. and *Phyllosticta Antirrhini* Syd.

The following is a description of the antirrhinum isolate (AI) upon its host: Pycnidia scattered, subepidermal, brown to black, ostiolate, slightly papillate, 100–140μ diam. occasionally up to 280μ. Pycnospores hyaline, cylindrical with rounded ends, frequently guttulate, 5·3–9·8 × 2·0–3·5μ, average 7·1 × 2·5μ, mostly without septa, 2–50% uni-septate, occasional spores bi-septate. Spores honey-yellow in mass.

Owing to the variable percentage of uni-septate spores in different collections of material, it appeared uncertain whether the fungus was *Diplodina Passerinii* or *Phyllosticta Antirrhini*. It was therefore decided to compare it with material of each fungus from the Kew Herbarium. According to Buddin & Wakefield (1924), the pycnospores of *Diplodina Passerinii* are two-celled and considerably larger than those of *Phyllosticta Antirrhini* when mature, although it is not easy to distinguish the fungi in the immature condition; measurements are not given. This difference was clearly illustrated in the Kew material; the presence of uni-septate spores and the greater size clearly differentiated the writer's material from the Kew specimens of *P. Antirrhini*, spores of which measured 3·0–5·0 × 1·5–2·0μ. The AI material, however, was comparable with the Kew material of *Diplodina Passerinii*. The Kew specimens of *D. Passerinii* Allesch. include material forming part of the herbarium of W. B. Grove and material contributed by W. Buddin. The specimens of Grove are all from naturally infected plants whilst those of Buddin fall into two classes; naturally infected and inoculated plants. The specimens were compared with the writer's own dried material obtained from both naturally infected and inoculated plants. Examined macroscopically, all the material appears to be very similar in form of lesion and distribution of pycnidia. The colour of the pycnidia varies from dark brown to black and the size also seems to be variable even on the same specimen. Pycnidia in the herbarium specimen collected at Kew by Grove are very dark in colour. The sizes vary from 100–300μ: the writer's material falls within these limits.

The best and apparently most mature of Grove's material is that collected by D. A. Boyd at Saltcoats, Ayrshire; in this, the spores are cylindrical with an average size of 7·8 × 2·8μ with a range in length of 6·0 × 9·1μ; about 80% are 1-septate. Some degree of variability is noticeable in other specimens collected by Grove, particularly with regard to the percentage of 1-septate spores present; this ranges from the material collected at Kew in which the spores are mostly non-septate to the Ayrshire material in which 80% are 1-septate.

The most noticeable feature of Buddin's specimens is the smaller size and the lower percentage of septate spores. Here again, considerable variation in the percentage of septate spores is observed, varying from less than 1 to 12%, whilst in the *Diplodina* leaf-spot of antirrhinum, produced by Buddin's strain, only occasional septate spores are observed. The spore-size in Buddin's naturally infected material collected at Slough is $6{\cdot}7 \times 2{\cdot}8\mu$ with a range in length of $5{\cdot}8$–$8{\cdot}0\mu$. The spore-size in the artificially infected material appears somewhat smaller, average lengths being 6·0, 6·3 and $6{\cdot}0\mu$ with a range of $5{\cdot}0$–$8{\cdot}5\mu$. In the leaf-spot specimen, the spore length averages $5{\cdot}5\mu$ with a range of $4{\cdot}5$–$6{\cdot}2\mu$. From this examination it appears that the spore-size is variable within wide limits.

The size of spores observed in the writer's fungus on the original host, although not consistently as large as that of the spores in Grove's Ayrshire specimen, falls within the range of measurements of spores observed in the specimens of Buddin. The size of the spores of AI when inoculated into godetia, var. Schamini, is larger and more comparable with the Ayrshire material of W. B. Grove, the average size being $8{\cdot}5 \times 3{\cdot}2\mu$ with a range in length of $7{\cdot}3$–$10{\cdot}0\mu$; approximately 25% of the spores are 1-septate.

From the above comparison it is suggested that the fungus obtained from antirrhinum in the present investigation is probably to be referred to *Diplodina Passerinii* Allesch. Other isolations have been obtained from Antirrhinum and these are the same as that which has been specially studied (AI).

Comparison with other isolates

The behaviour of the five fungi is essentially the same over a wide range of hosts into which they were inoculated. Except for the Cl and G isolates, the fungi are capable of becoming established on the host plant of each other. Although the G isolate is unable to infect antirrhinum, the GX isolate, however, also derived from the same host as G, does cause infection, thus suggesting that the godetia isolates are separate strains of the same fungus Cl also has not been successfully inoculated into antirrhinum. The host range, however, is so comparable that it is possible for all the fungi to be regarded as strains of the same species.

Material of each of the fungi upon their respective hosts and upon clarkia and godetia has been compared microscopically. In this comparison also, great similarity has been observed in the appearance of the disease developed as a result of inoculation with the various isolates. The similarity was most marked when the five fungi were inoculated into the same kind of plant.

In culture the fungi G, GX, Cl, SS and AI do not differ from one another sufficiently for them to be regarded as different species, and the degree of variability between them is no greater than that, e.g., between the various isolates of G or AI. It must be emphasized that the cultural characters of each of the five isolates when first obtained from their respective hosts were very similar; subsequently, however, considerable variations have occurred, variations which in many instances have been intimately associated with change in the degree of pathogenicity.

The above comparisons strongly suggest that the fungi are separate strains of a single species differing slightly in cultural behaviour and in pathogenicity. The species is provisionally determined as *Diplodina Passerinii* Allesch. Mr Buddin has kindly examined my material and agrees with this determination. He also informs me in a letter that in 1923 he obtained successful infection by inoculating *D. Passerinii* into stems of antirrhinums, and

that in some instances the fungus spread from the stems into the leaves, producing pycnidia thereon.

In the course of this investigation, other fungi, e.g. *Pythium* spp. and *Botrytis cinerea* have frequently been encountered killing young plants of godetia, antirrhinum, etc.

Historical

Diplodina Passerinii Allesch. was first described by Passerini (1891) under the name *Ascochyta decipiens*, which name, however, had been used previously by Trail for a fungus on Heliocharis. Allescher (1899) transferred both *A. decipiens* Pass. and *A. decipiens* Trail into the genus *Diplodina*, changing the specific name of the former to '*Passerinii*': the transference was made on the basis that *Diplodina* comprised stem-inhabiting fungi and *Ascochyta* the leaf-inhabiting fungi. He also records the fungus *Diplodina Antirrhini* Fautrey, which he regards as a synonym of *Ascochyta decipiens* Pass. Both species found on antirrhinum are recorded by Saccardo (1892). The work of Guba & Anderson (1919) on *Phyllosticta Antirrhini* Syd. makes clear the futility of segregating genera according to their occurrence on leaf or stem, and this is borne out by the results of the leaf and stem inoculations conducted by the writer. The fungus is described by Flachs (1931) under the name *Diplodina decipiens* Pass., probably due to the printer's error in Allescher's account in which the fungus is named *Diplodina decipiens*, *Passerinii*, nov.nom. without having the '*decipiens*' omitted as was obviously intended. The emended name of *Diplodina Passerinii* Allesch. is used in the accounts by Grove (1916, 1935) and Buddin & Wakefield (1924). The name *Ascochyta decipiens* Trail is retained by Grove for the fungus attacking *Heliocharis* with the comment that Allescher must have transferred it to the genus *Diplodina* without having seen it.

The following is the description from Saccardo (1892):

Ascochyta decipiens, Passer., Diagn. Fung. N.V., n. 39.

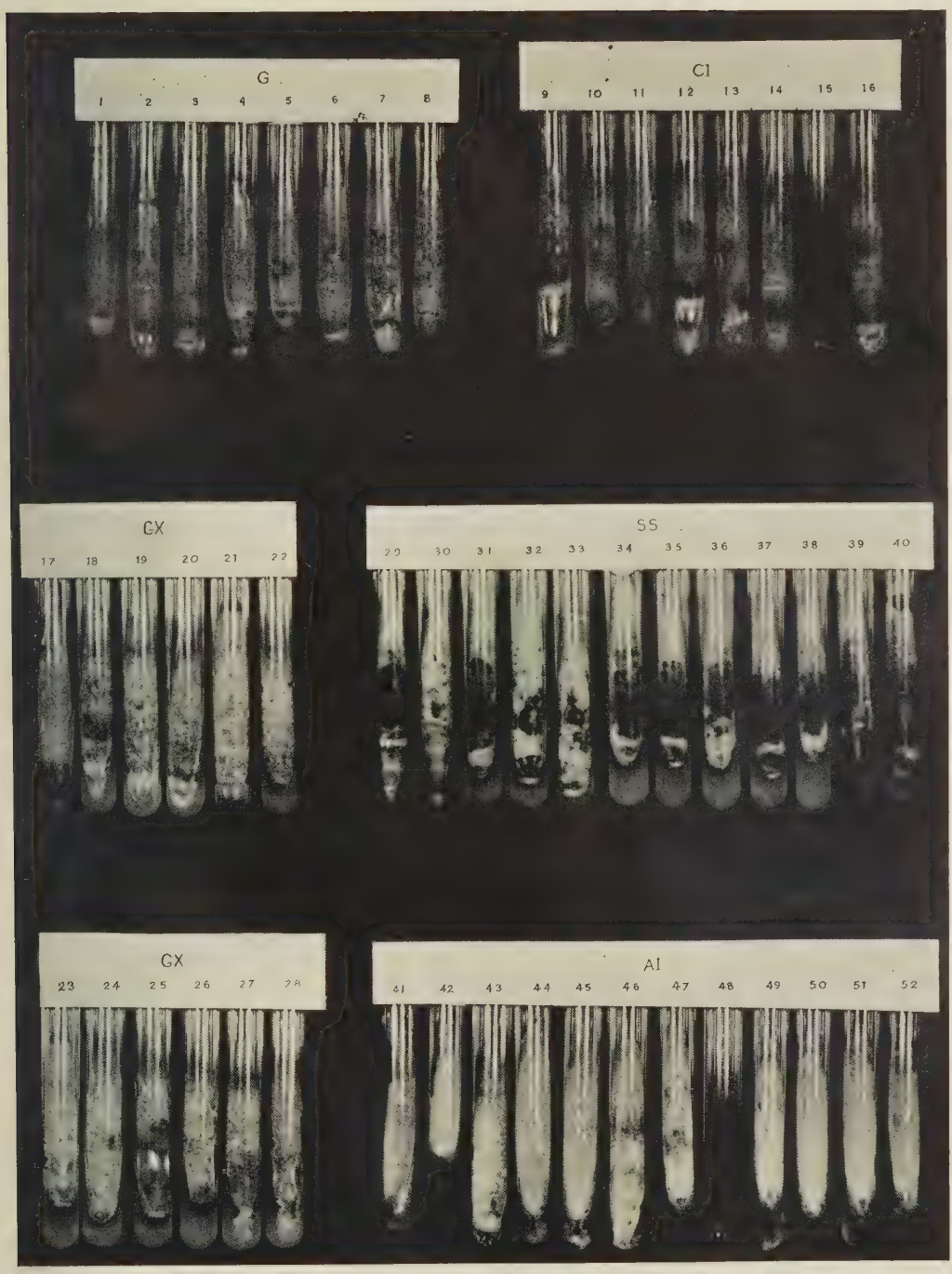
Peritheciis in matrice immutata sparsis vel laxe gregariis, minutis, epidermidem pustuliformiter sublevantibus, fuscis, contextu membranaceo-celluloso, flavo-fusco; sporulis cylindricis, utrinque obtusis, hyalinis, endoplasmate varie guttulato et obscure transversim diviso, prope medium septatis, non constrictis 12×3, raro $18–20 \times 3–4$. basidiis non visis.

Hab. in caule et ramis *Antirrhini majoris* ad moenia Parmae, Ital. bor.

Considerable discrepancy is evident between the measurements given by Saccardo (1892) 12×3, rarely $18–20 \times 3–4 \mu$, and those recorded by Grove (1935) $7–12 \times 2{\cdot}5–3 \mu$. Allescher (1899) quotes the same dimensions as Saccardo. In the present investigation the measurements by Grove have been accepted.

Summary

A wilt disease of godetia, clarkia, antirrhinum and sweet sultan (*Centaurea moschata*) caused by one or more species of pycnidial fungi is described. The fungi isolated from the various hosts have generally proved to be pathogenic to all of them when cross-inoculated. Other herbaceous ornamental plants are susceptible under experimental conditions. The various isolates are considered to be different strains of a single species which is provisionally identified as *Diplodina Passerinii* Allesch., previously recorded as a parasite of antirrhinum. The fungus is a wound parasite and gains entrance to the host through the stem, frequently just above the soil and at the leaf-axils. There is no evidence of infection via the roots. The cultural characters of the isolates are compared.



TAYLOR—A Wilt Disease of Godetias and other Ornamental Plants (pp. 91–101)

I wish to express my thanks to Prof. F. T. Brooks, F.R.S., who suggested and supervised the work, for his interest, advice and constructive criticism throughout the investigation and during the preparation of the manuscript. I am grateful to Miss E. M. Halley for kindly allowing me to use the results of her investigations and to Mr W. Buddin and Miss E. M. Wakefield for their assistance in the identification of the fungus.

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EXPLANATION OF PLATE 5

The five isolates, G, Cl, GX, SS, and AI growing on Dox's agar. For details see Table 4 in text (p. 97). Photo., 8 August 1940.

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THE IDENTIFICATION OF THE BACTERIA COMMONLY CAUSING SOFT ROT IN PLANTS

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THE commonest agents of soft rot of plants are certain species of bacteria which after gaining entry through wounds cause the separation of individual cells of parenchymatous tissue by enzymatic action. In Britain the most generally recorded bacterium causing soft rot is *Bacterium*[1] *carotovorum* (L. R. Jones) Lehm. (Lehmann & Neumann, 1927) from which *B. phytophthorum* (Appel) Burgwitz (Burgwitz, 1936) has been recently separated (Dowson, 1940) and is confined in nature, so far as is at present known, to the potato in which it causes blackleg. *B. aroideae* (Towns.) Stapp (Sorauer, 1928) has been recorded in this country attacking arum corms and hyacinth leaves (Moore, 1939), but in the United States has a wider range of host plants than *B. carotovorum* (Massey, 1924). *B. rhaponticum* Millard (Millard, 1924), the cause of the crown rot of rhubarb seems to be restricted to this host and has not been recorded outside Britain. To these four species of *Bacterium* recognized in this country must be added at least one member of the green fluorescent bacteria which compose the genus *Pseudomonas*.[1] *Ps. marginalis* (Brown) Stapp (Sorauer, 1928) infects lettuce under moist conditions through the stomata along the leaf edges to cause the disease known as marginal spot. Under very humid conditions such as are induced when the heart of a lettuce is stood in water, sprayed with a watery suspension of the organism and covered with a bell-jar, the marginal lesions develop rapidly into a typical soft rot affecting all tissues. When pure cultures of the above bacteria are inoculated into onion bulbs or cucumber the tissues are rotted in from 2–7 days and all except *B. rhaponticum* (Metcalfe, 1940*a*) produce similar effects upon inoculation into carrots, potato tubers and green stems of potato and tomato. The rot of potato tubers caused by inoculation with *Ps. marginalis* is characterized by the production of a buff colour and it is probable that some of the rotting of potato tubers in soil and in storage is due to this organism, as Bonde (1939), in the United States, showed that this kind of rot was due to a green fluorescent bacterium. It is also possible that *Ps. marginalis* is associated with *B. carotovorum*, *B. phytophthorum* and *B. aroideae* as green fluorescent colonies almost invariably arise on isolation plates of soft rot tissues but are discarded either because one or other of the last three bacteria is present, or because it is assumed that all green fluorescent colonies are those of the common saprophyte *Ps. fluorescens* (Flügge) Migula (= *Ps. aeruginosa* Schroeter). *B. lactis-aerogenes* Escherich (= *Aerobacter aerogenes* (Escherich) Beijerinck is also frequently associated with plant tissues, rotted or sound, and has been isolated from the older and deeply stained wood of the cricket bat

[1] The generic names, *Bacterium* and *Pseudomonas*, are used here in the sense advocated in my paper (*Zbl. Bakt.* II, 1939, **100**, 188) in which was given a new definition for the first so as to combine certain plant pathogens with the colon-typhoid group and the inclusion of the 'green fluorescent' plant disease producers with Migula's genus, *Pseudomonas*. Additional support for this procedure was advanced by Burkholder at the Third International Congress for Microbiology, New York, 1939 (*Report of Proceedings*, 1940, p. 163). The actual binomials used here are those first published, the references to which will be found at the end of this paper. What the original author meant by the genus *Bacterium* does not necessarily coincide with my definition (see, for instance, Millard's (1924) note on *B. rhaponticum*).

willow (Metcalfe, 1940*b*) primarily attacked by *B. salicis* (Day) Dowson, the cause of the watermark disease. Recently the author isolated the same bacterium from the innermost and unusually stained wood of elm attacked by the fungus *Ceratostomella ulmi* which causes Dutch elm disease. *B. lactis-aerogenes* is non-pathogenic and is not a cause of soft rot.

From the foregoing remarks it will be plain that isolations made from soft rot material may yield one or more bacteria capable of producing the disease upon inoculation and the investigator is faced with two problems, viz. exactly what soft rotting bacteria are present and which of them is responsible for the disease. Isolation plates nearly always yield green fluorescent colonies which are rarely tested for pathogenesis because another well-known bacterium, e.g. *B. carotovorum* is searched for first and, if found, is accused of being responsible for the rot under investigation. This is particularly true of potato tubers, the rot in which may be due to any one or all combinations of the following bacteria: *B. carotovorum*, *B. phytophthorum*, *B. aroideae* and *Ps. marginalis*. A complete diagnosis of a soft rot should involve the test for pathogenesis of all different colonies separately and in combination arising on isolation plates and the identification of all such as prove to be pathogenic. In what follows the different pathogenic and biochemical characters of the soft rot bacteria are described and also the methods for ascertaining such in the laboratory.

Procedure

(1) *Isolation*

Fragments of the rotted tissues are removed by means of a wire loop or forceps as aseptically as possible from the interior on the boundary between softened and sound tissue and transferred to tubes containing 5 ml. sterile water. In the course of 20 min. the bacteria will have diffused out into the surrounding water which will then be slightly turbid in appearance. A loopful of this suspension is streaked on to plates of bullock heart infusion agar of *p*H 6·8. From the colonies which appear in 2–3 days transfers are made from all which differ in appearance to slopes of the same medium thus establishing pure cultures of the different bacteria which can then be tested for pathogenesis.

(2) *Appearance of colonies of* Bacterium *spp.*

After 24 hr. incubation at 27° C., well-separated colonies of *B. carotovorum*, *B. aroideae* and *B. rhaponticum* are about 1 mm. diam.; those of the first are slightly irregular in outline, those of the two last are round. The colonies of *B. phytophthorum* are smaller, i.e. slower growing and round. After 48 hr. the colonies of all four are round, yellowish by transmitted light, opalescent by oblique light and dirty white by reflected light.

(3) *Appearance of colonies of* Pseudomonas *spp.*

The great majority of *Pseudomonas* species produce yellowish green colonies, round when first visible but later slightly irregular in outline, on plates of beef infusion agar (Bryan, 1927) into which the pigment fluorescin diffuses so that both plates and slope cultures take on a yellowish green colour.

(4) *Proof of pathogenesis*

Densely turbid suspensions are made from the above pure cultures by transferring a considerable amount of the bacterial growth to tubes containing 5 ml. sterile water and from these after shaking, loopfuls are transferred to the freshly cut surfaces of slices of onion or cucumber in Petri dishes containing plenty of water. At least one dish containing a slice of onion or cucumber must be left untouched as a control. Within 24 hr. at room temperature any of the five soft rot bacteria will have commenced to rot the onion or cucumber tissues. The rot is nearly complete in 3 days when caused by *B. aroideae*, *B. phytophthorum* or *Ps. marginalis*, but *B. carotovorum* and *B. rhaponticum* are slower in action and the rot produced by them is not complete until 5–6 days. If the control is unaffected it is reasonably certain that the rot appearing in the inoculated slices is due to the introduced bacterium and one may then proceed to its identification by the biochemical methods described below. Should

the control slice become rotted, thereby indicating the presence of soft rot bacteria on the onion or cucumber before slicing, the series must be discarded and a new lot commenced. The absence of rotting of the inoculated slices indicates that the introduced bacterium is non-pathogenic and can be discarded.

(5) *Identification of species of* Bacterium

If neither the well-separated colonies on isolation plates nor the surrounding liquid of the rotted slices are greenish it can be assumed that the bacteria concerned are members of the genus *Bacterium* and not *Pseudomonas*, and it now remains to determine the particular species present. This depends upon the action of the various species on maltose and xylose. Thus *B. carotovorum* does not produce acid in maltose, but forms both acid and gas in xylose when freshly isolated; *B. phytophthorum* forms acid and gas in both sugars when freshly isolated; *B. aroideae* produces acid in maltose but none in xylose, while *B. rhaponticum* forms only acid in both sugars. As a further confirmatory test for the last two, nutrient gelatine stab cultures should be made; *B. aroideae* liquefies gelatine, *B. phytophthorum* does not. From these facts the following key for identification has been constructed:

Medium	Reaction observed after 5 days	Species of *Bacterium*
(1) Maltose	No change in colour	*B. carotovorum*
	Acid (yellow) and gas	*B. phytophthorum*
	Acid (yellow) only	2 and 3
(2) Xylose	No change in colour	*B. aroideae*
	Acid (yellow) only	*B. rhaponticum*
(3) Gelatine	Liquefied	*B. aroideae*
	Not liquefied	*B. rhaponticum*

The bacteria to be tested are grown for 2 days in tubes of peptone water or 'bacto-tryptone' from which a loopful is introduced into each of three tubes containing maltose or xylose, a Durham fermentation tube and the indicator bromo-cresol-purple (*p*H 5·2 (yellow) to *p*H 6·8 (blue)) and incubated for 5 days at 25–27° C. The sugar media should be made up strictly as follows: 10 g. maltose or xylose is dissolved in a basal medium[1] containing $NH_4H_2PO_4$ 1·0 g., KCl 0·2 g., $MgSO_4.7H_2O$ 0·2 g., water 1000 ml., and 1 ml. of the indicator. The solutions are adjusted to *p*H 7 by the addition of NaOH of which about 6 ml. *N* soda will be required and distributed, 10 ml. per tube to each of which a Durham fermentation tube is added. The tubes thus filled are sterilized by bringing momentarily to 120° C. in the autoclave, turning off the gas and allowing to cool (Davis & Rogers, 1938). Both sets of tubes will then be pale mauve in colour.

(6) *Gas formation by species of* Bacterium

It has been pointed out by Harding & Morse (1909), Lacey (1926) and Bonde (1939) that the production of gas from the splitting of certain carbon compounds, notably sugars, by soft rot bacteria is variable in amount and may be absent, so that this character cannot be relied upon as specific. In my own investigations I have found that the ability to produce gas is soon lost or becomes diminished by both *B. carotovorum* and *B. phytophthorum* after they have been in artificial culture for a few months. On the other hand when freshly isolated both organisms produce gas from such compounds as xylose, dextrose, mannose, sucrose, lactose salicin and glycerol made up according to the method given above. When first isolated therefore a gas-former may be either *B. carotovorum* or *B. phytophthorum* but not *B. aroideae* which does not produce gas from such compounds. On the other hand old cultures of these three bacteria give similar reactions, i.e. acid and no gas, but *B. carotovorum* can always be recognized by its inability to form acid in maltose (Dowson, 1940) in which the other two produce acid. To distinguish between old cultures of *B. aroideae* and *B. phytophthorum* apart from the action in xylose recourse may be had to artificial inoculation of a host plant affected by only one of the bacteria. Tobacco stems or Arum (*Richardia*) lily petioles are suitable and are quickly rotted by *B. aroideae*, but not by *B. phytophthorum* when introduced by pricking with a needle charged with the growth from an agar slope culture.

(7) *Identification of* Pseudomonas marginalis

The only two species of *Pseudomonas* known to me to produce a rot when inoculated into onion or cucumber are *Ps. marginalis* and *Ps. syringae* van Hall (1902), which may be distinguished by their different reactions in sucrose. Thus *Ps. syringae* forms acid in sucrose (made up as described above

[1] *Manual of Methods*, Society of American Bacteriologists, II, 14–39.

with sucrose in place of maltose or xylose), while *Ps. marginalis* produces no acid. A practical means of distinguishing one species from the other is by artificial inoculation of suitable host plants. *Ps. marginalis* produces a very rapid rot of both potato tubers and lettuce heart, whilst *Ps. syringae* has but a slight effect on these two hosts.[1]

The biochemical characters of the soft rot bacteria considered in this paper and including the non-pathogenic *B. lactis-aerogenes* are summarized in the following table in which under the sugars − means no acid formed, A means acid, AG means acid and gas; under gelatine + means liquefaction and − means no liquefaction.

Species	Maltose	Xylose	Gelatine	Sucrose
B. carotovorum	−	AG	+	AG
B. phytophthorum	AG	AG	+	AG
B. aroideae	A	−	+	A
B. rhaponticum	A	A	−	A
B. aerogenes	AG	AG	−	AG
Ps. marginalis	−	A	+	−
Ps. syringae	−	A	+	A

Summary

Four species of *Bacterium* and one of *Pseudomonas* are capable of causing soft rot of plants in nature. *B. carotovorum* and *B. aroideae* have a wide range of natural hosts but *B. phytophthorum* and *B. rhaponticum* appear to be restricted to one specific host, potato and rhubarb respectively. *Ps. marginalis* recorded so far only from lettuce may be responsible for a certain type of potato tuber rot in soil or storage. The artificial host range is wide except for *B. rhaponticum* which produces a rapid soft rot only in onion and cucumber. The other four bacteria will also rot carrots, potato tubers and green stems of potato and tomato. *B. lactis-aerogenes* is frequently present in rotted or sound tissues but is not a cause of soft rot. The species of bacteria can be distinguished in the laboratory by certain biochemical reactions and by their different pathogenic powers which are described. The differentiating biochemical characters are summarized in tabular form.

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[1] It is probable that other members of the numerous green-fluorescent group of plant pathogens may produce rotting upon inoculation into hosts other than the two mentioned above. Since this paper was prepared the author has isolated from swedes a soft rotting bacterium corresponding in its biochemical characters with *Pseudomonas medicaginis* var. *phaseolicola* (Sackett) Burkholder, 1926.

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SOME PROPERTIES OF TOBACCO ETCH VIRUSES

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(With Plate 6)

Although the tobacco etch viruses were discovered 13 years ago and have since been reported as of economic importance in various parts of the world, their properties have been little studied. Johnson (1930) differentiated four strains on the basis of the symptoms they caused, and gave a list of susceptible hosts, all members of the Solanaceae. He found that severe etch virus was inactivated when infected leaves were dried. Chester (1935) stated that severe etch virus was serologically related to tobacco mosaic virus, but later found that this result was due to a contamination (1937) and that the etch viruses formed a serological group distinct from any other viruses. Stanley & Wyckoff (1937) were able to sediment severe etch virus from clarified sap by high-speed centrifugation; the rate of sedimentation was about the same as that of tobacco mosaic virus, but the boundaries were too diffuse for accurate measurements. Lauffer & Stanley (1938), without giving any further details of methods of preparation or other properties, placed this virus among those showing the phenomenon of anisotropy of flow. Kassanis (1939) showed that severe etch virus was transmitted by *Myzus persicae* Sulz.: he also found that it differed from any plant virus previously studied in that it caused the formation of crystalline intranuclear inclusion bodies.

The study of the properties of the etch viruses described in this paper was undertaken primarily in the hope that it would give some indication of the mechanism of formation of the specific intracellular inclusions. However, no differences have been found between these and other viruses that can account for their different behaviour in vivo. Two strains of tobacco etch virus were used; these were kindly supplied by Drs W. M. Stanley and W. C. Price of the Rockefeller Institute, Princeton, under the names severe etch virus (S.E.V.) and mild etch virus (M.E.V.) respectively.

Symptomatology

Quantitative infectivity tests with the etch viruses were made by local lesions, using a modification of the starch-iodine method devised by Holmes (1931) for tobacco mosaic virus. Five to seven days after inoculation the plants were placed in the dark from the afternoon to the next morning. The inoculated leaves were then picked, killed by being plunged into boiling water, decolorized with warm 95% ethyl alcohol, washed in tap water and treated with a solution of iodine in potassium iodide. Under good conditions the lesions now show clearly as black areas against a white background, even in leaves which previously showed no signs of lesions (Pl. 6, fig. 1). No definite time for leaving plants in the dark can be given, for it varies with the weather conditions controlling the photosynthetic activities of the plants. The form of the iodine lesions varies. Most often they are thick black rings with white centres, but sometimes they are well-defined solid spots and occasionally the spots have diffuse edges. The presence of the virus impedes the translocation of starch both

from the actual entry points and from other tissues invaded. Thus, by decolorizing and staining the inoculated leaves at various intervals after infection the path taken by the virus from the entry point is clearly shown. It first spreads slowly from the entry point to the nearest minor vein, down which it moves more rapidly to the main vein and the petiole; there is little or no movement in any other direction.

As the starch-iodine method is laborious and does not give good results unless the time for each treatment are exactly right, a search was made for a host that might give visi countable local lesions. This failed. None of the plants examined outside the Solanac took the virus, and *Solanum nodiflorum* and *S. Melongena* were also immune. *Hyoscyam niger*, *Datura Stramonium*, *Lycopersicum esculentum* and twelve species of *Nicotiana* were susceptible, but reacted to S.E.V. in much the same way as tobacco, var. White Burl Sometimes the rubbed leaves showed quite definite necrotic rings similar to the local lesi produced by the *S* strain of potato virus *X* (Salaman, 1938) (Pl. 6, fig. 2). More often tl showed faint, diffuse chlorotic spots that could not be counted accurately (Pl. 6, fig. and sometimes showed no obvious symptoms. It seemed that the type of local react depended on the weather conditions, but the exact conditions determining them were found.

The systemic symptoms of infection with S.E.V. are more constant. They appear fr 6 to 10 days after infection and first take the form of a clearing of the veins with a l necrotic etching. The symptoms first appear at the bases of the leaves and spread upwa (Pl. 6, fig. 4). The young, actively growing leaves usually soon have symptoms all over, some of the older leaves frequently have pronounced symptoms at the bases while the t look quite normal. In such leaves the external symptoms are correlated with the distribut of the virus, for no infection is obtained when the healthy looking parts of such leaves used as inoculum.

The vein clearing and etching persist for a few days and are then replaced by a gen pronounced chlorosis (Pl. 6, fig. 5). The subsequent growth of plants is much reduc Although the same number of leaves are produced as in healthy plants, they are m smaller, have a bright mottle and tend to lie horizontally while their margins turn downwa and inwards. The internodes are considerably shorter than usual, so that the whole p has a stunted appearance.

In the work on insect transmission the infected plants frequently produced gre distorted leaves. Sometimes the lamina developed on one side only of the main vein sometimes there was no appreciable lamina at all, when the leaf consisted of little but main vein and resembled a tendril. At first it was thought that this symptom was cau by a distinct strain of the virus, which had been separated from a mixture by the aphi but attempts to reproduce the symptoms by inoculation failed until small seedlings sim to those used in the insect work were infected. Then it was found that the deformatic a constant feature of S.E.V. infections in young seedlings.

The symptoms of mild etch take about 2 days longer to appear than those of severe e Inoculated leaves usually show diffuse chlorotic areas, and the first systemic symptoms a milder vein clearing and etching. When these symptoms fade they are replaced by a [illegible] interveinal mottle with green vein-banding, much less definite than the chlorosis of severe etch. Bawden (1939) suggested that the great stunting of plants infected with S.E.V. might be a result of the presence of nuclear abnormalities in the form of crystalline plates. However,

this now seems unlikely, for plants infected with M.E.V. are almost as large and well developed as healthy plants and yet their nuclei also contain plate-like inclusions.

Both types of intracellular inclusion, the cytoplasmic and intranuclear, described with S.E.V. (Kassanis, 1939) have been found constantly in plants infected with M.E.V., but they show some characteristic differences. Although, as with infections with S.E.V., all infected tissues contain inclusions, they are fewer. With S.E.V. the nuclei are often so packed with inclusions that they cannot be counted, whereas with M.E.V. the nuclei usually contain only one or two inclusions and the largest number ever found was eight (Pl. 6, fig. 6). The average size of the inclusions produced by M.E.V. is greater, the sides averaging about 8μ long instead of 5μ. Kassanis (1939) stated that the plates showed no extinctions in the polarizing microscope, and it was suggested that this might be because of their small size. When examined between crossed Nicol prisms the intranuclear inclusions formed by M.E.V. are clearly birefringent with straight extinction when viewed edgeways, but not when viewed flat. A more detailed examination of the intranuclear inclusions in plants infected with S.E.V. has now shown that these are similarly birefringent. Not all the intranuclear inclusions formed by M.E.V. seem to be flat plates, for many show a characteristic cross suggesting that they may be eight-sided bi-pyramids (Pl. 6, fig. 7). The larger size and more definite shape of the M.E.V. inclusions is probably a direct result of their smaller number. With S.E.V. it seems that many crystalline foci arise in each nucleus and the resulting crystals compete both for material and space, whereas with M.E.V. there are only a few foci from which crystal growth continues more slowly and free from competition.

Cytoplasmic inclusions of the *X*-body type are common in infections with M.E.V., although they may not occur in every cell as do the intranuclear inclusions. They are definitely granular, like those produced by S.E.V., but are smaller. The cytoplasmic inclusions are usually amorphous, but in the characteristically malformed leaves produced by infecting young seedlings with S.E.V. these inclusions tend to crystallize. This crystalline phase seems to be a secondary stage in the history of the *X*-bodies, and shows itself by the production within the granular bodies of needle-shaped, birefringent bodies varying in length from 2 to 10μ (Pl. 6, fig. 8). Whether these are true crystals or paracrystalline fibres, like those produced by precipitating tobacco mosaic virus with acid, is unknown. These birefringent needles have been seen in epidermal strips from malformed leaves within one month of infecting young seedlings, but they usually require longer than this and seem to develop as a result of ageing of the inclusion. All the *X*-bodies in a malformed leaf do not necessarily contain crystals of this type, but the greater the malformation the greater is the tendency to crystallize. Some parts of large *X*-bodies may contain these needles while other parts remain amorphous and granular. When epidermal strips are mounted in weak acid the needles disappear and the *X*-bodies regain their granular appearance. In addition to tobacco, such crystallized *X*-bodies have been found in plants of *Nicotiana glutinosa* and *Datura Stramonium*, which were infected with S.E.V. when seedlings and developed malformed leaves. *X*-bodies containing similar, though smaller, needles have also been found in leaves of tobacco plants that had been infected with M.E.V. for a long time, although they were not malformed, and in malformed leaves of *Nicotiana glutinosa* infected with M.E.V. A somewhat similar tendency of old *X*-bodies to crystallize has been reported by Henderson Smith (1930) and Sheffield (1934) with aucuba mosaic virus, but here the crystals are large plates and not small needles.

Immunological reactions and interactions with other viruses

To facilitate quantitative work and to study the possible relationships between S.E.V. and other plant viruses antisera were prepared against it. Two methods of immunizing rabbits were used. One rabbit received four intravenous injections at 5 day intervals of 2 c.c. of a concentrated virus preparation made by precipitation with ammonium sulphate, and a second rabbit received five intravenous injections of 2 c.c. of infective sap clarified by a few minutes centrifugation at 16,000 rev./min. The second rabbit showed signs of anaphylactic shock, but both produced antisera that precipitated at dilutions greater than 1/200.

Using the antisera at a dilution of 1/50 in 0·85 % NaCl solution good precipitin reactions were obtained with sap from plants infected with S.E.V., both in the crude state as expressed through muslin and after clarification by centrifugation. Crude sap usually reacted at dilutions down to 1/16, and sap clarified by the addition of Na_2HPO_4 and centrifugation reacted at 1/4 or 1/8. Preparations of S.E.V. made by precipitation with ammonium sulphate reacted at greater dilutions, sometimes at 1/256 or 1/512. With all the antigen preparations except crude sap, which gives a bulky green precipitate consisting largely of unspecific material, the precipitate has a fluffy open structure similar to that of the antiserum-precipitates formed by bacterial flagellar antigens and by viruses such as tobacco mosaic and potato *X*, which have asymmetrical particles and show the phenomenon of anisotropy of flow.

S.E.V. crude and clarified sap, and in concentrated preparations, has been tested against antisera to tobacco mosaic virus, potato viruses *X* and *Y*, and *Hyoscyamus* virus 3. It failed to precipitate with any in similar conditions to those in which it reacted with its own antiserum. Similarly, preparations of these four viruses failed to precipitate with S.E.V. antiserum while reacting with their homologous antisera. This confirms Chester's (1937) second statement that the etch viruses are a serologically distinct group. We are unable to confirm his further statement that S.E.V. was a poor test antigen whereas M.E.V. was a good one. We made many attempts to obtain precipitin reactions between S.E.V. antiserum and crude and clarified saps from plants infected with M.E.V., but all failed. The most likely cause of this failure seemed to be a too small content of M.E.V. in infective sap to give a visible reaction, so attempts were made to concentrate the virus by precipitations with ammonium sulphate. Although this method had worked well in providing antigen preparations with S.E.V., it failed to give preparations of M.E.V. that would precipitate with S.E.V. antiserum. It then seemed possible that M.E.V. and S.E.V. might be serologically unrelated in spite of the fact that both gave such similar intracellular inclusions, and a rabbit was therefore immunized with clarified sap from plants infected with M.E.V. It was given five intravenous injections of 2 c.c. each and then, as these produced increasingly severe signs of anaphylactic shock, three further intraperitoneal injections of 6 c.c. each. The serum produced in this manner also failed to precipitate with crude or clarified sap from mild etch plants or with preparations made by precipitations with ammonium sulphate. That the serum contained specific antibodies, however, was clearly shown when it was tested against preparations of S.E.V., for then precipitates were obtained exactly as if antiserum prepared against S.E.V. itself had been used. The serum was weaker than those produced against S.E.V. giving a titre of only 1/16. Thus it seems clear that S.E.V. and M.E.V. are serologically related virus strains, that is, they contain common antigenic groupings, for when either is injected into rabbits antibodies are produced with which S.E.V. unites and precipitates. The most probable explana-

tion of their different serological behaviour is a quantitative one; whereas S.E.V. occurs in sufficient concentration in infective sap to precipitate as well as to cause the production of antibodies, the concentration of M.E.V. is sufficient to cause the production of antibodies but not to give a visible precipitate when mixed with them. This view is supported by the fact that comparative infectivity tests between sap from plants infected with S.E.V. and M.E.V., both by local lesions and infection end-points, showed the former to have at least four times the virus content of the latter.

To test further the relationship between M.E.V. and S.E.V., plants infected with the former were reinoculated with S.E.V. Healthy control plants inoculated at the same time developed typical symptoms of severe etch, but none infected with M.E.V. did. These tests were done in the winter, and the protection afforded by the M.E.V. was not complete, for the young actively growing leaves showed a slight increase in mottling, but nothing comparable with the severe disease produced in the plants infected with S.E.V. alone. In experiments done during the summer in better growing conditions protection was more complete, and plants previously suffering from mild etch showed no increase in symptoms when reinoculated with S.E.V. Thus by the plant protection test, as well as by serological methods, S.E.V. and M.E.V. can be classified as related strains. The S.E.V. was obviously able to enter and move through plants already suffering from mild etch, for the young leaves of the reinoculated plants, even when they showed no increase in symptoms, contained this virus, which could be detected by further inoculations to healthy plants. The basis of the protection seems to lie in the presence of the M.E.V. preventing the multiplication of the S.E.V. sufficiently for it to produce its characteristic symptoms. This was indicated by the fact that the virus content of the reinoculated plants did not increase significantly above that of plants infected with M.E.V. alone. It was insufficient to give a precipitate with antiserum and its infectivity, as indicated by starch lesions, was the same as that of normal mild etch sap.

As a further test of possible relationships between S.E.V. and other viruses, plants infected with tobacco mosaic virus, potato viruses *X* and *Y*, and Hyoscyamus virus 3 were reinoculated with S.E.V. All of them became fully infected. The external symptoms produced by Hyoscyamus virus 3 resemble to some extent those of severe etch, so the appearance of these plants altered only slightly, but the others developed the typical severe etch symptoms. Microscopical examination showed the presence of the characteristic intranuclear inclusions in all four kinds of reinoculated plants. Sap taken from the young leaves of these plants one month after reinoculation reacted with S.E.V. antiserum as strongly as that taken from plants infected with S.E.V. alone, showing that the presence of these viruses, in contrast to M.E.V., had no effect on the multiplication of S.E.V.

An unexpected result was obtained with the plants infected with potato virus *Y* (P.V.Y.) and Hyoscyamus virus 3 (HY.V.3). Sap from plants infected with either of these viruses alone reacts with its homologous antiserum at dilutions of from 1/8 to 1/16, indicating that their virus content is much the same as that of severe etch. One month after the plants had been reinoculated with S.E.V., however, sap taken from young leaves failed to react with antiserum to the virus with which the plants were originally infected, i.e. with either HY.V.3 or P.V.Y. antiserum, and reacted only with S.E.V. antiserum. The behaviour of sap from the plants infected with potato virus *X* and then reinoculated with S.E.V. was quite different, for this reacted with both S.E.V. and potato virus *X* antisera; similarly, sap from the reinoculated tobacco mosaic plants reacted with both S.E.V. and tobacco mosaic virus antisera. One

possible explanation of this result was that HY.V.3 and P.V.Y. were present in the saps of the reinoculated plants but were unable to react with their respective antisera in the presence of S.E.V. To test this, sap was taken from plants infected separately with these two viruses and mixed with sap from either healthy plants or plants infected with S.E.V. These mixtures were then tested serologically. The mixing with healthy sap slowed down the rate of precipitation, but the S.E.V. sap had no additional effect, both HY.V.3 and P.V.Y. reacting with their antisera in its presence. Thus the result with the saps of the reinoculated plants strongly suggested that S.E.V. was able to suppress and replace P.V.Y. and HY.V.3, even in plants in which they had become well established. As such a phenomenon had not previously been recorded, further experiments were made to test the possibility in greater detail.

First, the effect on the virus content of plants by inoculating them with mixtures of viruses was tested. Healthy plants were inoculated with the following seven viruses or mixtures of viruses, the mixtures consisting of equal quantities of crude sap from plants infected with the specified virus: (1) S.E.V., (2) HY.V.3, (3) P.V.Y., (4) S.E.V., P.V.Y., (5) S.E.V., HY.V.3, (6) S.E.V., P.V.Y., HY.V.3, (7) HY.V.3, P.V.Y. The plants rubbed with (1), (4), (5) and (6) all developed typical symptoms of severe etch, those rubbed with (2) and (7) developed symptoms typical of infection with HY.V.3, and only those rubbed with (3) showed the vein-banding characteristic of infection with P.V.Y. Three weeks after infection, sap was expressed from each batch of plants, clarified by a few minutes centrifugation at 16,000 rev./min., and tested against antisera. The summarized results are given in Table 1, and show that the presence of S.E.V. in the inoculum prevented both HY.V.3 and P.V.Y. from multiplying sufficiently to give a precipitin reaction, while the presence of HY.V.3 similarly prevented the multiplication of P.V.Y. Minute amounts of S.E.V. in the inoculum are sufficient to prevent the multiplication of HY.V.3. In one experiment crude sap from severe etch plants was diluted in sap from plants with HY.V.3, and at all dilutions up to 1/1000, i.e. almost the dilution end-point of S.E.V. in water, the plants developed severe etch, and their sap failed to react with antiserum to HY.V.3.

TABLE 1. *Precipitin reactions of saps taken from plants inoculated with severe etch virus, Hyoscyamus virus 3 and potato virus Y alone and in mixtures*

Antigen: Sap from plants inoculated with	Antiserum: S.E.V.	HY.V.3	P.V.Y.
S.E.V.	+	−	−
HY.V.3	−	+	−
P.V.Y.	−	−	+
S.E.V., P.V.Y.	+	−	−
S.E.V., HY.V.3	+	−	−
S.E.V., HY.V.3, P.V.Y.	+	−	−
HY.V.3, P.V.Y.	−	+	−

+ indicates specific precipitate; − indicates no precipitate.

To test the specificity of this reaction tobacco plants were also inoculated with S.E.V., potato virus *X*, and tobacco mosaic virus, both alone and in mixtures. After 3 weeks, clarified sap from these plants was titrated against antisera to the three viruses (see Table 2). Although the precipitin titres of tobacco mosaic virus and potato virus *X* are much greater than that of S.E.V., there is no significant difference between the titres of these viruses whether they are in a plant alone or in combination. Similarly, when the sap of plants infected with

both potato viruses *X* and *Y* was titrated against antisera, the titre with virus *Y* antiserum was the same as that of sap from plants infected with virus *Y* alone, and the titre with virus *X* antiserum the same as that given by control plants infected with virus *X* alone.

As all workers may not accept the precipitin titres as an accurate index of the virus content of sap, a further test was done with the mixtures containing tobacco mosaic virus to see if the presence of potato virus *X* or S.E.V. reduced the number of lesions caused by tobacco mosaic virus. Saps taken from plants infected with the virus mixtures were compared, on opposite half-leaves of *Nicotiana glutinosa* at various dilutions, with sap from control plants infected only with tobacco mosaic virus. The results are given in Table 3, and agree with the serological results in showing no significant difference between the content of tobacco mosaic virus in the various plants.

TABLE 2. *Precipitin titres of saps taken from plants inoculated with severe etch virus, potato virus X and tobacco mosaic virus alone and in mixtures*

		Antiserum						
		S.E.V.		P.V.X.			T.M.V.	
Antigen	Dilution of sap	1/4	1/8	1/50	1/100	1/200	1/400	1/800
S.E.V.		++	+	−	−	−	−	−
P.V.X.		−	−	++	+	−	−	−
T.M.V.		−	−	−	−	−	++	+
S.E.V., P.V.X.		++	+	+++	++	+	−	−
S.E.V., T.M.V.		++	+	−	−	−	++	+
S.E.V., P.V.X., T.M.V.		++	+	++	+	−	++	+
P.V.X., T.M.V.		−	−	++	+	−	++	+

+ indicates specific precipitates; − indicates no precipitate.

TABLE 3. *Infectivity of saps from plants infected with mixtures of severe etch virus, potato virus X, and tobacco mosaic virus compared with that of sap from plants infected with tobacco mosaic virus alone*

	Average number of lesions per leaf at		
Virus mixture	1/100	1/1000	1/10,000
T.M.V. + S.E.V. / T.M.V.	22·7 / 22·3	26·2 / 25·1	1·9 / 1·8
T.M.V. + P.V.X. / T.M.V.	29·3 / 36·5	3·6 / 4·1	0·4 / 0·5
T.M.V. + P.V.X. + S.E.V. / T.M.V.	31·7 / 46·6	4·7 / 5·9	1·3 / 2·7

Ten half-leaves were inoculated for each test. The numerator is the average number of lesions caused by the virus mixture and the denominator the average number caused by the T.M.V. control on the opposite halves of the same leaves.

From these results it seems clear that it is not a general result for the presence of one virus to restrict the multiplication of another in a plant. The ability of S.E.V. to do this to HY.V.3 and P.V.Y., and of HY.V.3 to inhibit P.V.Y., suggests some kind of relationship between them. This effect has been confirmed in many separate experiments, both in which plants have been infected with mixed inocula and in which plants previously infected with one or other of these viruses have been reinoculated with S.E.V. The only occasions when S.E.V. has failed to supplant these viruses has been in the winter in bad growing conditions when plants infected with HY.V.3, and already in a wilted condition, were reinoculated.

The replacement of P.V.Y. in actively growing leaves by S.E.V. and HY.V.3 can be followed

clearly by testing sap serologically at various intervals after reinoculation. This is shown in Table 4. Plants that had been infected with P.V.Y. for 13 days, when they showed typical symptoms and their sap reacted at 1 in 4 with virus *Y* antiserum, were divided into three. One set was left as a control, another inoculated with S.E.V., and the third inoculated with HY.V.3. At 5, 9, 15 and 21 days samples were taken from young leaves, their saps expressed and tested for their precipitation with antisera (Table 4).

When fairly large plants are infected with S.E.V. only the young, actively growing leaves develop symptoms, the mature leaves remaining apparently healthy and virus-free. Similarly, if plants that have been infected with P.V.Y. for some time are reinoculated with S.E.V. only the young leaves develop symptoms of etch, the old ones retaining the mild vein-banding symptoms. When sap is taken from such plants and tested serologically, that from the upper leaves reacts with S.E.V. antiserum and not with virus *Y* antiserum, whereas that taken from the older leaves reacts with virus *Y* antiserum and not S.E.V. antiserum.

TABLE 4. *Change in serological reaction when plants infected with potato virus Y are reinoculated with severe etch virus or Hyoscyamus virus* 3

		Antiserum											
		P.V.Y.				S.E.V.				HY.V.3			
Dilution of antigen ...		1/1	1/2	1/4	1/8	1/1	1/2	1/4	1/8	1/1	1/2	1/4	1/8
Antigen	Time												
P.V.Y., control	5	+++	++	+	−	−	−	−	−	−	−	−	−
	9	++++	+++	++	+	−	−	−	−	−	−	−	−
	15	++++	+++	++	+	−	−	−	−	−	−	−	−
	21	++++	+++	++	+	−	−	−	−	−	−	−	−
Reinoculated with S.E.V.	5	+++	++	−	−	−	−	−	−	−	−	−	−
	9	−	−	−	−	−	−	−	−	−	−	−	−
	15	−	−	−	−	++++	+++	++	+	−	−	−	−
	21	−	−	−	−	++++	+++	++	+	−	−	−	−
Reinoculated with HY.V.3	5	+++	++	+	−	−	−	−	−	−	−	−	−
	9	+++	++	+	−	−	−	−	−	−	−	−	−
	15	−	−	−	−	−	−	−	−	+++	++	+	−
	21	−	−	−	−	−	−	−	−	++++	+++	++	+

The time is given as days after inoculation. + indicates specific precipitate; − indicates no precipitate.

Whether the presence of S.E.V. suppresses P.V.Y. and HY.V.3 completely, or only greatly reduces them, cannot be stated definitely, although the former seems more probable. Sap from reinoculated plants, and from plants inoculated with virus mixtures, has been precipitated with ammonium sulphate, a treatment known to concentrate P.V.Y. and HY.V.3 (Bawden & Pirie, 1939), and then tested against antisera. Although this treatment concentrated the S.E.V. eight times, for the precipitin titre went up from 1/8 to 1/64, the preparations still failed to react with antisera to P.V.Y. or HY.V.3. Similarly, attempts to demonstrate these two viruses in the young leaves of plants inoculated with them and S.E.V. by means of aphides have failed. A large number of transmissions have been made from such leaves but all of them gave S.E.V. alone. That some infections with P.V.Y. or HY.V.3 might have been expected if they were present in these leaves is indicated by the fact that when leaves were rubbed over their whole surfaces with mixtures of S.E.V. and these, P.V.Y. and HY.V.3 could frequently be recovered by means of aphides. Thus from plants infected with such mixtures, aphides fed on the inoculated leaves sometimes transmitted S.E.V. and sometimes either P.V.Y. or HY.V.3, but from the leaves that became infected by systemic spread they transmitted only S.E.V.

PROPERTIES IN VITRO

In all the tests made S.E.V. behaved very like P.V.Y. and HY.V.3.

Dilution end-point. Infections with S.E.V. were rarely obtained at dilutions of crude sap greater than 1/5000, and with M.E.V. the dilution end-point was usually around 1/1000. Stanley & Loring (1938) found that the virus content of turkish tobacco was nearly twice that of *N. glutinosa*. We have found no such difference between White Burley tobacco and *N. glutinosa*. When saps from plants of these two species, which had been infected for the same length of time, were tested on opposite half-leaves for their infectivity over a range of dilutions they gave no significant differences, and they gave the same precipitation end-point when tested serologically.

Thermal inactivation point. Heating crude S.E.V. sap for 10 min. at 58° C. rendered it non-infective, and heating for this time at temperatures between 52 and 58° C. greatly reduced infectivity. M.E.V. was completely inactivated by 10 min. heating of crude infective sap at 54° C.

Longevity. After 5 days' storage at room temperature the infectivity of S.E.V. sap had fallen to less than 1/100 of the original, but complete inactivation was not obtained until 13 days. In picked leaves left to dry at room temperature, the virus survived a little longer, but was inactive after 24 days. Sap from plants with mild etch was completely inactive after 8 days at room temperature. Loss of infectivity on heating or ageing is a gradual process, and it is to be expected that the time required for complete inactivation will be affected by virus content. It is probable that the differences in the thermal inactivation points and longevities of S.E.V. and M.E.V. are a reflexion of their differing concentrations rather than of differences in stability.

Filterability. S.E.V. in sap clarified by centrifugation passed through a Pasteur-Chamberland L_1 filter candle, but not through L_3. Attempts to filter the virus through L_3 and L_5 candles were made at various *p*H values, but all failed, although it was found that adjusting the *p*H to 7 or higher greatly facilitated the passage of the virus through beds of diatomaceous earth.

Effect of trypsin. When trypsin was added to preparations of S.E.V. their infectivity was immediately reduced, the reduction being proportional to the amount of trypsin added. When incubated with trypsin under conditions in which the enzyme is proteolytically active no further fall in activity occurred. The fall in infectivity on mixing was not reflected by a similar fall in the serological activity, which also remained constant during incubation. Thus S.E.V. behaves towards trypsin like all other plant viruses that have been tested, with the exception of potato virus *X* (Bawden & Pirie, 1939). The enzyme does not hydrolyse the viruses, but its presence in the inoculum greatly reduces infectivity.

Partial purification. S.E.V. is readily absorbed on to other materials, and any treatment causing a large precipitate in infective sap also causes loss of virus. The precipitates that separate when acid or alcohol is added carry with them all the virus, that caused by freezing carries most of it, and the least loss during clarification of crude sap is produced by the addition of sufficient Na_2HPO_4 to cause flocculation. The instability of S.E.V., coupled with this loss by absorption, makes its rigorous purification extremely difficult by precipitation methods, but partially purified and greatly concentrated preparations can be made by the following method. After clarification by the addition of Na_2HPO_4 and centrifuging, the infective sap is two-fifths saturated with ammonium sulphate. The brown precipitate is spun off, suspended in a volume of water equal to about one-fifth of the original sap, brought to *p*H 7, and centrifuged to free from insoluble material. The supernatant fluid is one-fifth saturated with ammonium sulphate and centrifuged. The precipitate contains some of the virus, but most remains in the supernatant fluid, which is then two-fifths saturated with ammonium sulphate and again centrifuged. This precipitate is taken up in water, adjusted to about *p*H 7·8 with alkali, and then incubated for 3 hr. with 0·3 % trypsin. After centrifuging, the preparation is again one-fifth saturated with ammonium sulphate and the precipitate formed removed by centrifugation. The virus is precipitated from the supernatant fluid by the addition of more ammonium sulphate, spun off and dissolved in water. Using this method preparations have been made which showed definite anisotropy of flow, and which reacted with antiserum up to dilutions of 1/512. They were always brown and contained too much impurity for analytical figures to be of any value as an indication of the chemical nature of the virus. However, the preparations behaved so similarly to those of P.V.Y. and HY.V.3 (Bawden & Pirie, 1939) that it is reasonable to assume that S.E.V. is also a nucleoprotein with asymmetrical particles. As with these two viruses, it seems that the processes of purification used for S.E.V. lead to a decrease in infectivity without a corresponding loss of serological activity, for these highly active antigenic preparations were always much less infective than crude infective sap. Also, on storage their infectivity rapidly declined whereas their serological activity decreased much more slowly. This

method failed to produce concentrated preparations of M.E.V., probably because of the smaller virus content of sap, which would make the losses by absorption on the discarded precipitates so much more important.

Discussion

The main point arising from this work that calls for discussion is the relationship between the etch viruses and potato virus *Y* and Hyoscyamus virus 3. The two etch viruses are clearly strains of the same virus, related to one another in the same manner as are, for example, tobacco mosaic and aucuba mosaic viruses, for they contain common antigens and plants infected with one are protected against infection by the other. The relationship of S.E.V. with P.V.Y. and HY.V.3, however, seems to differ from any previously recognized. The three viruses are transmitted by insects in precisely the same manner, falling into the group called 'non-persistent' by Watson & Roberts (1939), and have essentially similar physico-chemical properties. Yet they are not serologically related and plants infected with P.V.Y. or HY.V.3 are not protected against S.E.V. They cannot, therefore, be regarded as related strains in the sense in which the phrase is at present applied to viruses. However, the fact that plants infected with S.E.V. are protected against infection with P.V.Y. or HY.V.3, and plants with HY.V.3 are protected against P.V.Y. suggests some fairly close relationship between the three, for when any of these is transmitted to plants already infected with a virus with quite dissimilar properties, for example, potato virus *X*, it enters and multiplies as in healthy plants. If words coined for use with higher organisms can legitimately be applied to viruses, M.E.V. and S.E.V. can probably be regarded as recently derived varieties of a single species, and the etch viruses, P.V.Y. and HY.V.3 as separate species of the same genus. It is possible that other viruses of the non-persistent group, e.g. cucumber virus 1 and potato virus *A*, which also have similar properties in vitro, will be found to be related to S.E.V., P.V.Y. and HY.V.3 in the same manner.

In previous work on the ability of one virus to protect plants against infection with others, serological methods could not be used, for only serologically related viruses were known to interact in this manner. The work mainly consisted in demonstrating that a virus causing a mild disease protected plants against serologically related viruses causing more severe symptoms, although that the protection is reciprocal has occasionally been shown (Bawden, 1939). If either one of a pair of such related viruses is established in a plant the other is unable to develop, but if the two are inoculated together to a healthy plant both usually enter and multiply, the resulting symptoms being a compromise between those caused by the two viruses acting alone. The interactions of S.E.V. and M.E.V. provide another illustration of this well-known phenomenon. The interactions of S.E.V., P.V.Y. and HY.V.3 differ from those of virus strains in three ways. First, the protection is not reciprocal, for although plants already infected with S.E.V. are protected against the other two, plants infected with these are not protected against the more severe disease caused by S.E.V. Secondly, when these viruses are introduced into plants together with S.E.V. they are unable to multiply detectably and the resulting symptoms are those of severe etch. Thirdly, even in plants in which they are well established they are suppressed and replaced by S.E.V. The mechanism underlying the protection afforded to a plant against one strain by previous infection with another is not yet established, but it is generally believed to be because the strains either utilize the same materials or the same sites for reproduction, and if one is already fully established the other is unable to multiply. Two possible explanations offer themselves for

the different interactions between S.E.V., P.V.Y. and HY.V.3. Infection with S.E.V. may give rise to conditions in the plant that inactivate the other two viruses, although the viruses have such similar stabilities in vitro that it is difficult to imagine conditions which would inactivate these without also inactivating S.E.V. Alternatively, these three viruses may be as unstable and have as short an existence in vivo as in vitro, and in the plant they may be continually being destroyed, the content of active virus being kept reasonably constant by the continual production of new virus. Then, if these viruses are produced from the same materials or at the same sites, but have different affinities for these materials or sites, with S.E.V. having the greatest and P.V.Y. the least affinity, these results can be interpreted. For when plants are inoculated with mixtures of the viruses, the constituent with greatest affinity will multiply more rapidly and usurp materials or sites needed by the others; and when plants already infected with a virus of small affinity are reinoculated with one of greater affinity, the original infecting virus will be replaced by the second as it becomes inactivated. The differences between the reciprocal protection afforded by strains of one virus and the interactions between these three serologically unrelated viruses can be interpreted on the basis that strains both utilize and have the same affinities for the same 'virus-making' sites or materials, whereas these viruses utilize the same materials but have different affinities for them.

Although no appreciable differences have been found between the properties of S.E.V., P.V.Y. and HY.V.3 in vitro, they differ widely in their behaviour in the plant. S.E.V. causes the production of large numbers of inclusions in the nuclei and in the cytoplasm; HY.V.3 causes large numbers of cytoplasmic inclusions, but none has been found in plants infected with P.V.Y. Bawden & Sheffield (1939) suggested that the inclusions formed by tobacco mosaic and related viruses might be insoluble complexes formed by the union of the viruses with host constituents. They could not suggest whether these constituents were normal plant products or abnormal ones produced as a result of virus activity. The three insect-transmitted viruses precipitate in such similar conditions in vitro that it is to be expected that any normal plant constituent which precipitates one would also precipitate the others. Therefore, if the view be adopted that the inclusions formed by S.E.V. and HY.V.3 are also insoluble virus-host complexes (a view supported by Sheffield's (1941) discovery that the inclusions are infective and chemically complex), it seems probable that the substances with which the viruses unite are not normal host constituents, but products specific to the particular infection. The different external symptoms caused by the three viruses show that they have different physiological activities and attack different materials in the plant; the different cytological pictures may be a further indication of these differences.

Summary

The symptoms of severe and mild etch are described; plants suffering from either contain both intranuclear and cytoplasmic inclusions. Fewer and larger crystals are formed in the nuclei of plants with mild etch. Plants infected as seedlings with S.E.V. develop malformed leaves, in which the cytoplasmic inclusions crystallize to give rise to birefringent needles. M.E.V. protects plants against infection with S.E.V.; the two are serologically related, for antisera prepared against either reacted with S.E.V. Precipitation was not obtained with M.E.V., presumably because the virus content of sap is too small.

Although not serologically related to potato virus *Y* or Hyoscyamus virus 3, S.E.V. has similar properties in vitro and is transmitted in the same way. The interactions of these

three viruses in the plant suggests that they are related. Plants infected with either of the other viruses are not protected against S.E.V., and those infected with P.V.Y. are susceptible to HY.V.3. Plants infected with S.E.V., however, are protected against the other viruses, and those infected with HY.V.3 are protected against P.V.Y. S.E.V. is able to suppress these two viruses when healthy plants are infected with a mixed inoculum, and to supplant them in tissues in which they are already established. Similarly, HY.V.3 can suppress and supplant P.V.Y. Possible interpretations are given for these results. S.E.V. has asymmetrical particles, for concentrated preparations showed anisotropy of flow.

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EXPLANATION OF PLATE 6

Fig. 1. Tobacco leaf inoculated with S.E.V., decolorized and stained with iodine. Before treatment the leaf showed no sign of local lesions.

Fig. 2. Tobacco leaf inoculated with S.E.V. showing local lesions of the necrotic ring type.

Fig. 3. Tobacco leaf inoculated with S.E.V. showing diffuse chlorotic local lesions.

Fig. 4. Young leaves of plants recently infected with S.E.V., showing vein clearing and necrotic etching. Note the symptoms spreading from the bases to the tips.

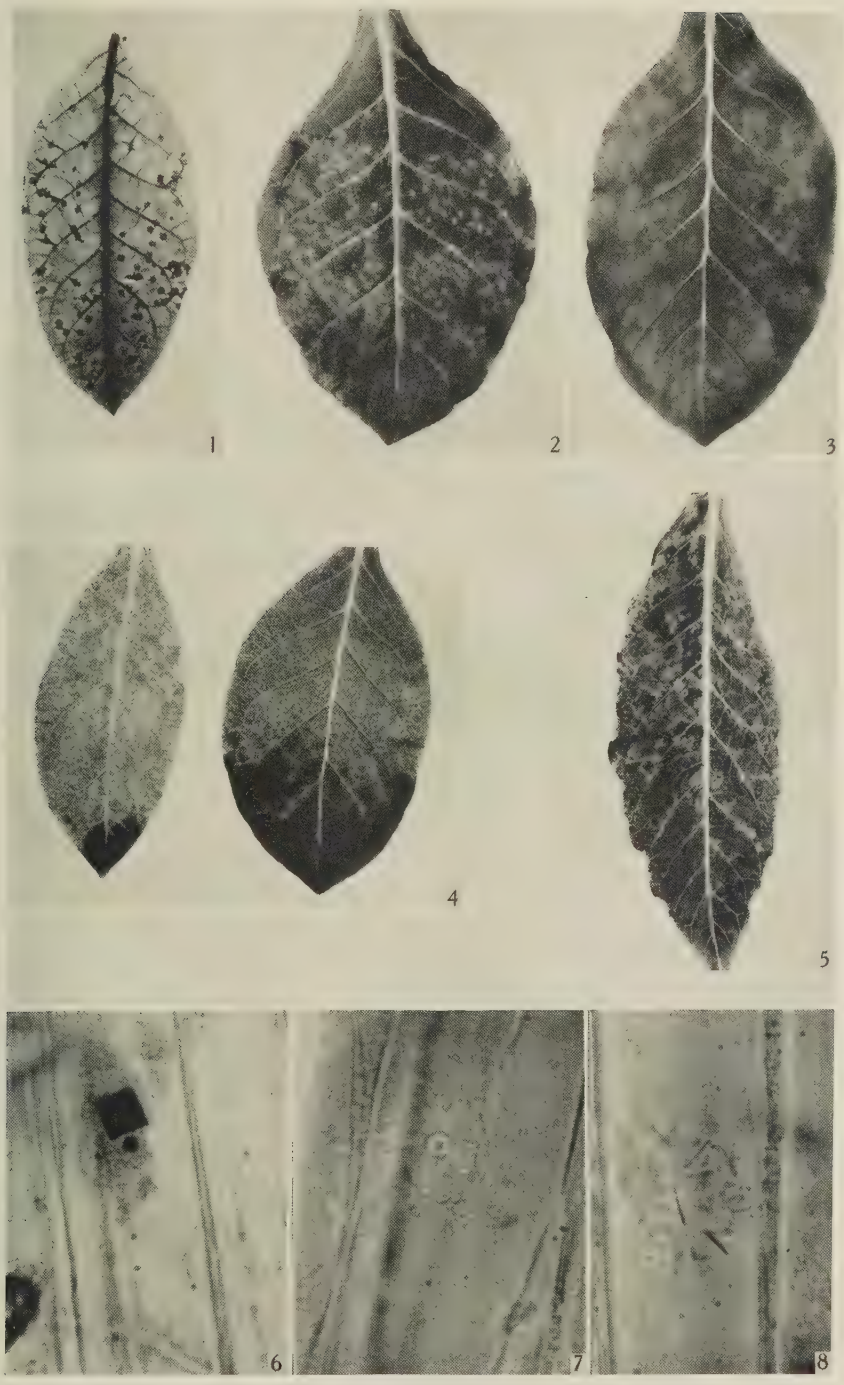
Fig. 5. Leaf from a plant infected with S.E.V. for a month showing a general yellow mottling.

Fig. 6. Epidermal cell from tobacco leaf infected with M.E.V., fixed in formol saline and stained with haematoxylin, showing a nucleus containing a single large crystal and the nucleolus. × 900.

Fig. 7. Living epidermal cell from tobacco plant infected with M.E.V. showing a crystal with a definite cross suggesting a bi-pyramid shape. × 900.

Fig. 8. Epidermal living cell from a malformed leaf of a tobacco plant infected with S.E.V. when a seedling. The cytoplasmic inclusion has crystallized and contains needles that are birefringent. × 900.

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BAWDEN AND KASSANIS—SOME PROPERTIES OF TOBACCO ETCH VIRUSES (pp. 107–18)

THE OVER-WINTERING OF *MYZUS PERSICAE* SULZ. ON BRASSICAE IN NORTH WALES

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Davies (1934) considered hibernation of Apterae on winter Brassicae to be the most important method of over-wintering of *Myzus persicae* Sulz. He says: 'It was soon apparent that over-wintering eggs of *M. persicae* on peaches and nectarines could only account for a mere fraction of the alate spring migrants that arrive on potatoes throughout the country during late May and June.' Davies did not leave complete records, but he evidently found *M. persicae* on Brassicae during December (1931) and 'late winter' and 'early spring' particularly on savoy cabbage (*Brassica oleracea* var.) and he says: 'The numbers of *M. persicae* found on Brassicae of various kinds in early spring, leaves no doubt concerning the main source of alate spring migrants.'

Heinze & Profft (1938), in Germany, state that Viviparae occur on Brassicae until mid-February, but cannot be found on them later in the season. Theobald (1926) states that he found eggs of *M. persicae* on Brassicae. As far as the author has been able to discover, there have been no subsequent observations to support this, and no Sexuales have been found on Brassicae during the present investigation.

Observations on the over-wintering of *M. persicae* in North Wales made by Thomas in 1937–8 are summarized below:

Number of M. persicae *Sulz. per* 100 *leaves on Brassicae in the winter of* 1937–8
(*all centres averaged*)

1937	1938
27 Oct. —190	27 Jan. —13
29 Oct. —108	7 Feb. —10
11 Nov.— 47	11 Feb.— 0
24 Nov.— 63	28 Mar.— 0
	30 Mar.— 0
	1 Apr.— 0
	12 Apr.— 0

These observations appeared to be in line with those of Heinze & Profft (1938), and Thomas suggested that it was desirable to make more detailed observations, paying particular attention to Brassicae in private gardens in sheltered areas.

Field observations, 1938–9, 1939–40

(i) *At the College Farm, Aber, Caernarvonshire*

(*a*) *Field crops* (*swedes and cabbage*). Observations were started on 13 September 1938 and carried on until all the cabbages were cut, and all the swedes lifted (Table 1). The importance of these crops as over-wintering host plants is negligible unless migration of the small numbers of Alatae found during the winter on to garden crops and field crops of spring cabbage, cauliflowers, etc., occurs before the crops are used. That such winter

migration is possible, is indicated by the fact that on 12 November 1939 large numbers of aphides were observed flying in the Bangor district, and smaller numbers on other days about this time. It is thought, however, that such migration must be of infrequent occurrence. Kale was examined at the same centre but no *Myzus persicae* was found on it in 1938–9. Spring cabbage, cauliflowers and broccoli are rarely grown on a large scale in the western part of North Wales.

TABLE 1. *Numbers of* M. persicae *Sulz. on swedes and cabbage at the College Farm, Aber, 1938–9, and 1939–40 (per 100 leaves)*

Date	Swedes (*B. Napus* L.)	Cabbage (*B. oleracea* var.)
13 Sept. 1938	134 (4 Alatae)	198 (2 Alatae)
14 Oct. ,,	40	10
9 Nov. ,,	2	214 (4 Alatae)
22 ,, ,,	18	1000 (2 Alatae)
28 ,, ,,	6	234 (6 Alatae)
5 Dec. ,,	48 (4 Alatae)	330 (4 Alatae)
12 ,, ,,	36	196
19 ,, ,,	72	102
28 ,, ,,	177	All cut
2 Jan. 1939	239	—
9 ,, ,,	109	—
16 ,, ,,	30	—
23 ,, ,,	18	—
6 Sept. ,,	700 (8 Alatae)	1456 (8 Alatae)
27 ,, ,,	632 (22 Alatae)	332 (4 Alatae)
17 Nov. ,,	44 (6 Alatae)	390 (16 Alatae)
7 Dec. ,,	24	724 (22 Alatae)
22 ,, ,,	34 (2 Alatae)	191 (34 Alatae)
8 Jan. 1940	18	74 (4 Alatae)

TABLE 2. *Number of* M. persicae *Sulz. per 100 leaves* on several varieties of garden Brassicae, 1938–9 and 1939–40*

Date 1938	*M. persicae* Sulz. per 100 leaves	Date 1939	*M. persicae* Sulz. per 100 leaves
19 Dec.	350	3 Apr.	158
28 ,,	180	11 ,,	55 (2 Alatae)
		19 ,,	212
1939		26 ,,	188
2 Jan.	140	3 May	372
9 ,,	158	10 ,,	112
16 ,,	106	23 ,,	277
23 ,,	44	3 June	570 (2 Alatae)
30 ,,	70		
6 Feb.	42	1939–40	
16 ,,	128	21 Nov. 1939	94 (2 Alatae)
20 ,,	224	12 Dec. 1939	57
27 ,,	78 (2 Alatae on 10 leaves, savoy cabbage)	8 Jan. 1940	26 (1 Alata)
		4 Mar. 1940	0
6 Mar.	124	21 ,, 1940	0
13 ,,	406		
23 ,,	202		

* These figures in most cases are calculated from counts on a smaller number of leaves.

(*b*) *Garden Brassicae.* In the garden observations were not started until 19 December 1938. The following varieties of Brassicae were examined: savoy cabbage, spring cabbage, broccoli, kale, purple sprouting broccoli and brussels sprouts (Table 2). It is important to note in connexion with these figures that old savoy cabbage plants were left in the ground

until June; they would normally have been lifted in March. In these counts nymphs with wing buds were found on practically every occasion.

(ii) *Other centres*

It was not possible to keep such detailed records as those at Aber at any other centre. Nevertheless, evidence was obtained showing that *M. persicae* over-wintered as Apterae on winter Brassicae in most North Wales districts in 1938–9. Tables 3 and 4 are records for centres in the extreme eastern part of Denbighshire and for Madryn in south Caernarvonshire respectively. No *M. persicae* was found at Madryn after 9 March 1939 until the spring migration in late May and June, but at Nevin, about 4 miles away, several adult Apterae were found on purple sprouting broccoli on 17 May. This was considerably earlier than any spring migrants were noticed, so it seems probable that *M. persicae* hibernated as Apterae in this district.

TABLE 3. *Observations at Holt, Denbighshire (field conditions)*

Date 1939	No. of *M. persicae* Sulz. per 100 leaves
13 Feb.	Spring cabbage 28; brussels sprouts 8
17 Mar.	Spring cabbage 14; savoy cabbage 192
18 Apr.	Spring cabbage 122 and 516 (two fields)
19 May	Spring cabbage 3510; cauliflowers 36
6 June	Cauliflowers 220
16 June	Cauliflowers 480

TABLE 4. *Observations at Madryn, south Caernarvonshire. Number of* M. persicae *Sulz. per 100 leaves*

Date	Swedes	Garden
6 Sept. 1938	57	—
21 Oct. 1938	7	Savoy cabbage 52; brussels sprouts 72
2 Nov. 1938	43	Savoy cabbage 40; brussels sprouts 108
16 Feb. 1939	—	Savoy cabbage 12; brussels sprouts 0
9 Mar. 1939	—	Savoy cabbage 8

Records of M. persicae *Sulz. at other centres*

(F) = field conditions. (G) = garden conditions

5 Sept. 1938. 44 per 100 leaves on swedes at Aberdaron, south Caernarvonshire. (F)
21 Oct. 1938. 50 per 100 leaves on swedes at Aberdaron, south Caernarvonshire. (F)
15 Feb. 1939. 270 per 100 leaves on savoys at Bangor, Caernarvonshire. (G)
16 Feb. 1939. Calculated 3000 on 20–30 tubers, seed potatoes in glasshouse at Glanconway, Denbighshire.
6 Mar. 1939. 60 per 100 leaves on savoys at Llysfasi, Denbighshire. (G)
17 May 1939. Several adult Apterae on purple sprouting broccoli at Nevin, south Caernarvonshire. (G)
18 May 1939. In fair numbers on great bindweed, Bangor. (G)

Records for 1940

See Table 2 for Aber records

26 Feb. 1940. No *M. persicae* could be found at Madryn, Caernarvonshire. (G)
14 Mar. 1940. A thorough search failed to reveal any *M. persicae* on savoys in the Holt district. (F)

Influence of host plant

An outstanding characteristic of *M. persicae* is its preference for the older leaves of the host plants. In the case of the potato crop, work in North Wales has shown conclusively that *M. persicae* prefers the lower leaves of the plant (data not published). A few records indicating the distribution on savoy cabbage which had run to seed are given in Table 5.

Table 5. *Distribution of* M. persicae *Sulz. on savoy cabbage.* (*Number of aphides per* 10 *leaves*)

Date 1939	Top leaves	Middle leaves	Bottom leaves
11 Apr.	0	—	14
19 ,,	0	30	70
26 ,,	0	16	58
3 May	0	25	108
10 ,,	17	0	25
23 ,,	11	27	64
3 June	2	4	207

Table 6. *Analysis of indices given in Table* 2. *Numbers of* M. persicae *Sulz. per* 10 *leav*

Date 1938	Savoy cabbage	Brussels sprouts	Broccoli	Purple sprouting broccoli	Kale	Spring cabbage
19 Dec.	112	3	3	23	—	—
28 ,,	26	9	4	32	—	—
1939						
2 Jan.	42	5	0	10	—	—
9 ,,	45	17	1	13	3	—
16 ,,	24	0	2	23	1	—
23 ,,	22	0	0	0	0	—
30 ,,	25	0	0	10	0	—
6 Feb.	19	0	0	2	0	—
16 ,,	27	0	0	37	0	—
20 ,,	95	0	0	17	0	—
27 ,,	38	0	0	1	0	—
6 Mar.	59	3	0	0	0	—
13 ,,	151	—	0	46	0	6
23 ,,	74	—	0	27	0	0
3 Apr.	75	—	1	0	0	3
11 ,,	14	—	0	8	0	13*
19 ,,	70	—	5	1	0	12*
26 ,,	58	—	—	1	—	2*
3 May	108	—	—	16	—	8*
10 ,,	25	—	—	3	—	18*
23 ,,	64	—	—	9	—	26*
3 June	207	—	—	3	—	19

* These figures refer to ten whole plants.

This habit of *M. persicae* is in very marked contrast to that of the aphis *Brevicorynebrassic* Linn. which also occurs on winter Brassicae, but takes up a position as near the heart or the plant as possible, and, as a result is affected to a smaller extent by adverse weather conditions.

The varieties of cabbage examined were as a rule more successful as winter hosts than other kinds of Brassicae. The erect-leaved and tall varieties, such as broccoli, kale and brussels sprouts, were not as a rule successful over-wintering hosts (Table 6). Reference to Table 1

shows the very marked difference between swedes and cabbage which were grown in an exposed field at Aber. After stormy weather in this instance, the aphides on swedes were almost entirely found on what had been the lee side of the plants.

The type of leaf seems to be of considerable importance. Davies (1934) pointed out the importance of savoy cabbages, the crinkly leaves of which offer shelter to the hibernating aphides, and this has been confirmed in the present work (see Tables 3, 4 and 6). In addition it has been found that the curled leaves of purple sprouting broccoli offer good shelter (Table 6), but that the smooth-leaved varieties are less suitable. The most important requirements for an over-wintering host plant seem to be a low growth habit and the possession of crinkly or curly leaves, both of which conditions offer shelter to the hibernating aphides.

Influence of weather

Insufficient data were obtained to allow of any detailed discussion of the influence of weather on the population of apterous *Myzus persicae* Sulz. hibernating on winter Brassicae. The following observations were made.

(i) *In field and garden conditions*

Rain and storms which either washed or blew the aphides off the plants and injured them had an adverse effect on the numbers, while during a single very dry frosty period the numbers increased. From 18 to 23 Dec. 1938 there was a spell of hard frost, and it will be seen from Table 1 that the numbers of aphides increased from 72 to 177 per 100 leaves on swedes. (Table 2 is misleading, since only a small number of leaves was taken on each variety, and the aphides were very unevenly distributed on the varieties.) On the other hand, there was heavy rain and wind between 2 and 23 Jan. 1939 with a thunderstorm on 15 Jan., and the number of aphides on swedes fell from 239 to 18 per 100 leaves. The numbers in the garden also fell and kept low until 16 Feb., the weather in the meantime being wet and stormy on several days. The very severe weather conditions of January 1940 which caused severe damage to all brassica plants seen in several localities of North Wales probably killed most of the *M. persicae* in this area. In addition to the records given in the tables, other garden crops and weeds were searched without success. According to Davies (1934) wilting causes *M. persicae* to fall off the plants.

(ii) *In an experimental plot; movement of Apterae*

In the autumn of 1938 about forty broccoli plants varying from 6 to 15 in. in height were planted in a plot in the College garden and some of them were inoculated with apterous viviparous females of *M. persicae*. It was found in the field, however, that broccoli was not a successful host plant owing probably to the erect and smooth nature of the leaves, and the results obtained in this experiment are thus of little value. The weather had the same effect as in the field, and there was some indication that aphides shaken off the plants during storms, etc., reappear in small numbers on other plants and thus spread the infestation of the crop. No evidence of voluntary migration was obtained.

Plants originally free from aphides were found to be infested by Apterae after storms on the following occasions: 28 Nov. 1938 (one plant), 6 Dec. 1938 (one plant), 10 Jan. 1939 (two plants), 15 Feb. 1939 (two plants), 26 Feb. 1939 (one plant), 23 Mar. 1939 (two plants).

Each of the original inoculations in the plot died out and in no case was there any definite evidence that hibernation occurred. The nearest approach was in the case of a plant inoculated in December which remained infested.

Infestations of potatoes and the over-wintering of *M. persicae* Sulz. on Brassicae

From the limited number of observations it is probably not safe to conclude that high infestations on potatoes in summer follow seasons when *M. persicae* over-winters in large numbers on Brassicae, and vice versa. On the other hand, the observations suggest that this is so. In 1938 infestations of *M. persicae* on potatoes were low and this followed a winter

when the aphis was difficult to find on Brassicae. In 1939 potato infestations were considerably higher following high infestations in winter Brassicae. As far as could be observed, no *M. persicae* over-wintered on Brassicae in North Wales in the season 1939–40, and this was followed by a summer (1940) when potato infestations were at a record low level.

Summary

1. No evidence was obtained to support Theobald's (1926) statement that *M. persicae* Sulz. lays eggs on winter Brassicae. Considerable support for Davies's (1934) views has been obtained, and his statements regarding the importance of the different host plants, particularly savoy cabbage, have been confirmed.

2. The 1937–8 observations suggested that *M. persicae* Sulz. did not over-winter on field Brassicae; particular attention, however, was not paid to Brassicae in gardens, and it is possible that a few aphides over-wintered on savoys in sheltered places. In 1938–9 considerable numbers over-wintered both under field and garden conditions, savoys being the favourite host plant. It must be emphasized that in the garden under observation the savoys were left in the ground considerably longer than they would have been left normally.

3. Conditions were very different in the winter of 1939–40, and after the hard frost in January none could be found out of doors in any situation. This work suggests, therefore, that in North Wales *M. persicae* Sulz. over-winters readily as Apterae on Brassicae under favourable conditions, but that in some seasons none succeeds in over-wintering out of doors.

4. In North Wales, peaches as a winter host are probably of minor importance, but glasshouses form a reservoir from which numerous *M. persicae* Sulz. may migrate in spring and early summer.

5. From the detailed studies of populations of *M. persicae* Sulz. on potato crops in North Wales it seems probable that years of high infestations follow winters when widespread hibernation on Brassicae has occurred; and vice versa, years of low infestation on potato follow years when few or no *M. persicae* over-winter on winter Brassicae.

The author is indebted to the Agricultural Research Council for a grant which covered the expenses involved in carrying out this investigation. He is also indebted to Dr I. Thomas for suggesting this line of work and for some of the records quoted.

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A STUDY OF THE FREQUENCY WITH WHICH HONEYBEES VISIT RED CLOVER (*TRIFOLIUM PRATENSE*), TOGETHER WITH AN EXAMINATION OF THE ENVIRONMENTAL CONDITIONS

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(With 4 Text-figures)

The honeybee plays an important role in the pollination of fruit, vegetable and other crops. This was demonstrated during the years immediately following 1918 when fruit crops in particular were very poor, after the epidemic of acarine and other bee diseases had reduced the population of honeybees in the country by at least 90%. There is little doubt that the honeybee is of much greater value to the nation as a pollinator than as a honey producer, and it is considered by many authorities to be the most valuable of the insect pollinators. An estimation of its value as compared with other insects as a pollinator of various crops is important, so that steps may be taken to increase the population in those localities where there is a deficiency or where the presence of bees is most desirable.

The present paper is concerned with the disputed question of the value of the honeybee as a pollinator of red clover grown for seed. Much work has already been done, notably in America and Holland, in determining the relative value of various insects as pollinators of red clover, a good example of a self-sterile leguminous plant. Little work has, however, been carried out in the British Isles, and it is desirable that observations made in other countries should be repeated and extended in Britain since climatic factors may greatly influence the pollination of plants both by affecting the plants themselves and also the insects responsible for their pollination. The present work was carried out during 1939 and 1940 in two large fields of mixed varieties of red clover growing near Harpenden on a heavy clay soil, the nearest hives being about 400 yd. away.

The availability of red clover pollen to the honeybee

Parker (1925) showed that the pollen of various plants becomes available to visiting insects at different, but usually definite, times of the day, e.g. the pollen of alsike clover (*Trifolium hybridum*) is available to the honeybee at Ames, Iowa, from 8 a.m. to 6 p.m., whereas from white sweet clover (*Melilotus alba*) pollen is available only during the middle of the day and the early afternoon. Information is needed as to the times of the day at which the pollen of red clover (*Trifolium pratense*) is available to insects in this country, since the honeybee only works under certain environmental conditions, and these conditions and the availability of pollen must coincide to some extent if it is to play any part in the pollination of this plant.

To determine at what time of day the pollen of this plant becomes available for pollination, use was made of the fact that individuals of many species of bumble-bee can be found working red clover throughout the hours of daylight. On a number of days, bumble-bees

were captured in the clover field, their pollen loads, if present, removed and the insect immediately released and kept under close observation for as long as possible. In this way it was possible to determine whether or not the particular insect was collecting pollen, by seeing whether pollen was loaded into the pollen baskets while the insect was flying from flower to flower and whether the pollen load increased in size. Frequently the bumble-bee

TABLE 1. *The times of day during which pollen was collected from red clover* (Trifolium pratense) *by bumble-bees at Harpenden, Herts, on* 16 *June* 1940

No. of bumble-bee	Time under observation (G.M.T.)	Whether collecting pollen or not as determined by: Behaviour	Whether collecting pollen or not as determined by: Increase in size of load
1	7.15 a.m.– 7.27 a.m.	–	–
2	7.30 – 7.39	–	–
3	7.45 – 8.2	–	–
4	8.15 – 8.22	+?	–
5	8.26 – 8.44	+?	+?
6	9.4 – 9.21	+?	+?
7	9.29 – 9.35	+	+?
8	9.42 – 9.53	+	+
9	10.5 –10.15	+	+
10	10.48 –11.17	+	+
11	11.20 –11.26	+	+
12	11.47 –12.3 p.m.	+	+
13	12.20 p.m.–12.33	+	+
14	12.37 –12.44	+	+
15	12.46 –12.59	+	+?
16	1.5 – 1.8	+	+?
17	1.16 – 1.31	+	+
18	2.10 – 2.14	+	+?
19	2.17 – 2.39	+	+
20	3.23 – 3.42	+	+
21	4.5 – 4.16	+	+
22	5.0 – 5.18	+	+
23	5.25 – 5.46	+	+
24	5.51 – 6.15	+	+
25	6.24 – 6.37	+	+
26	6.42 – 7.0	+	+
27	7.3 – 7.21	+	+
28	7.26 – 7.44	+	+
29	7.47 – 8.1	+	+?
30	8.9 – 8.25	+	+?
31	8.37 – 8.42	+	+?
32	8.50 – 9.10	+	+?
33	9.23 – 9.38	+	–?
34	9.47 – 9.52	+?	–
35	10.0 –10.7	–	–
36	10.15 –10.34	–	–
37	10.42 –10.46	–	–

on being released after removal of its pollen load flew away and could not be kept under observation. Table 1 shows the results of a typical day's observation. Red clover pollen was available for collection from about 9 a.m. to 8.30 p.m. (G.M.T.) on 16 June 1940. It is probable that pollen was only available in the right condition for transference to the bee's body during this period, otherwise pollen would automatically have been collected at other times by the nectar-seeking bumble-bees.

Other observations were made by noting throughout the day the number of bumble-bees out of ten observed on each occasion in the clover field, carrying out the movements indicative of the transference of pollen to the pollen baskets. Two series of observations were

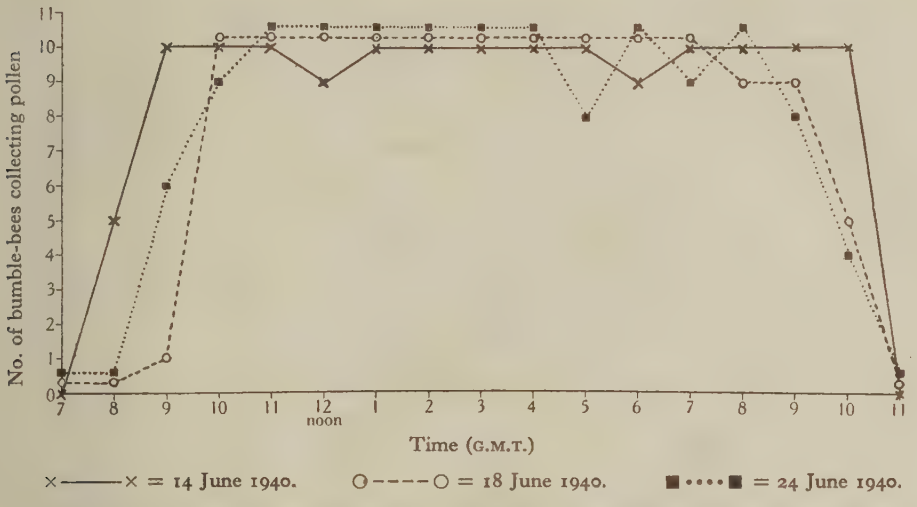


Fig. 1. No. of bumble-bees, in a group of 10 observed on each occasion, collecting red clover pollen on 3 days in June 1940

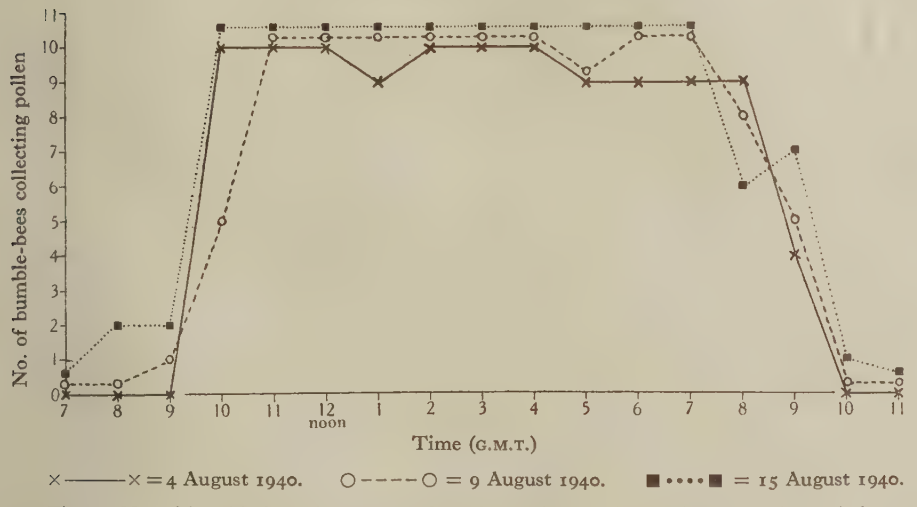


Fig. 2. No. of bumble-bees, in a group of 10 observed on each occasion, collecting red clover pollen on 3 days in August 1940

made, (1) during the first crop of flowers in June, and (2) in August when the second crop of flowers were in bloom, the first having been cut and harvested. The results, expressed in Figs. 1 and 2, are in close agreement with those of Table 1.

The collection of red clover pollen by the honeybee

Parker (1926) concluded that, with clovers, except red clover, the pollen is simply collected as a supplementary load while the bee is gathering nectar, and that no special effort in the collection of the pollen is made except to pack it into the pollen baskets and carry it to the hive. With *Trifolium pratense*, however, the bees merely treated this clover as a source of pollen. This does not accord with the work of Dunham (1939), Armstrong & Jamieson (1940) or many other observers (e.g. Valle, 1935; Stapel & Erikson, 1936), which showed that the honeybee does on occasion visit red clover flowers for nectar as well as for pollen. That the honeybee visits even first crop red clover for nectar appears to be supported by the following observations.

The honeybee when going deliberately to collect pollen from a plant carries honey from the hive to moisten the pollen in order to facilitate packing in the pollen baskets; and, when gathering pollen from a plant which has both nectar and pollen available, it invariably uses nectar from the plant itself with which to moisten the pollen. A simple test was applied to honeybees and bumble-bees working red clover at various times to determine whether the contents of the honey-stomach were honey or nectar, i.e. whether the collection of pollen was incidental to the collection of nectar or deliberate. The bee was killed, the contents of the honey stomach absorbed on to filter paper, and the outline of the liquid on the filter paper pencilled in while the drop was still moist. After drying at room temperature for 12 hr., honey leaves a translucent spot, while the nectar spots vary in degree of translucency from almost none to about half that of honey, depending upon the density of the nectar. By comparison with test spots it was relatively easy to decide whether a particular sample was nectar or honey. Of the 484 honeybees examined 277 were carrying nectar, 185 were carrying honey and 22 cases were doubtful, 19 of the latter not containing sufficient fluid for a proper test to be made. Similarly, of 204 bumble-bees examined, all except three which had almost empty honey stomachs, were carrying nectar. It may therefore be concluded that in the Harpenden district during 1940 the honeybee worked red clover for nectar (60%), pollen being collected only incidentally in these cases, and also deliberately for pollen (40%).

Size of load of red clover pollen carried by the honeybee and number of flowers visited

In order to obtain a fairly accurate estimate of the number of pollen grains collected by the honeybee on one foraging trip, bees carrying red clover pollen were collected as they entered the hive. The pollen loads were carefully removed from the hind legs of each bee and placed in glycerine. The grains were counted in suspension in glycerine in a Thoma cell after having been prepared by Dunham's method (1939*b*) except that the pollen grains were stained with fuchsin instead of brilliant green. In each instance a check was made to ensure that the grains were those of red clover. During these experiments the bees were chiefly working red clover and white Dutch clover, the pollen grains of which are easily distinguishable when stained in fuchsin and mounted in glycerine gelatine. The results of these counts are given in Table 2, which shows that the honeybees carried on the average approximately 284,000 red clover pollen grains per load, whereas Dunham (1939*b*) found the average figure was about 347,500 grains. Dunham also gave figures for the number of

pollen grains contained in unvisited flowers and flowers pollinated by the honeybee. These averaged respectively 1153 and 148, clearly showing the thoroughness of the honeybee in pollen collecting.

TABLE 2. *Number of pollen grains of red clover carried by honeybee*

No. of bee	Total no. of red clover pollen grains in pollen baskets	No. of bee	Total no. of red clover pollen grains in pollen baskets
1	224,000	16	304,000
2	630,000	17	198,000
3	281,000	18	282,000
4	127,000	19	291,000
5	312,000	20	147,000
6	280,000	21	132,000
7	149,000	22	513,000
8	184,000	23	264,000
9	300,000	24	500,000
10	471,000	25	138,000
11	218,000	26	286,000
12	292,000	27	292,000
13	283,000	28	283,000
14	287,000	29	317,000
15	310,000	30	211,000

The minimum pollinating activity of the honeybee in collecting an average load of red clover pollen can be estimated by dividing the average number of pollen grains per bee by the number of pollen grains which the bees removed from the pollinated flower, i.e. approximately 1000 grains. Making use of Dunham's figures for the number of grains in pollinated and unpollinated flowers one obtains the figure of 284 for the number of flowers visited by each bee in collecting a load of pollen, whereas Dunham obtained a figure of 348. Data obtained by Dunham (1939*b*) indicated that approximately 12·5 red clover flowers were visited per minute. This is in close agreement with the results obtained from about fifty observations made at Rothamsted where on the average fourteen flowers were visited per minute. By dividing the total number of flowers visited in collecting a pollen load by the rate of pollinating activity, a figure of 20·3 is obtained, which represents the number of minutes required to collect an average load of pollen. This is close to the figure of 27·8 min. obtained by Dunham. To check this, experiments were made with marked bees. A bee alighting on the hive entrance with a load of red clover pollen was marked with a quick-drying cellulose enamel and immediately liberated. When the bee left the hive again the time was noted and also the time of return if the bee was carrying, as was usually the case, another load of red clover pollen. It was only possible to make twenty-three observations of this kind, and the average length of time taken by the bee from leaving to returning was approximately 24·2 min. Making allowance for the distance to and from the clover field (approximately 400 yd.) and for the fact that the bee might be fatigued from its previous journeys, this accords well with the figure of approximately 20 min. estimated from the previous observations.

CONSTANCY OF THE HONEYBEE WHEN WORKING RED CLOVER

Numerous observations have been recorded which show the remarkable constancy with which honeybees visit flowers of a single species on any one trip. In order to make certain that this constancy is exhibited by the honeybee when working red clover, bees working

this plant were captured immediately on their return to the hive and their pollen loads removed and subjected to pollen analysis. Individual smears made of these pollen loads were each washed three times with absolute alcohol to remove substances of a fatty nature adhering to their surfaces, stained for 5 min. at room temperature in acid fuchsin in absolute alcohol, rewashed in absolute alcohol and mounted in glycerine-gelatine. Prepared in this way most of the pollen grains assume the fully expanded state with their walls clearly demarcated by the fuchsin, and there is little danger of confusing them with pollen grains from another species. The pollen loads of 100 bees were examined in early August in this way, and, only in seven were *any* grains other than those of red clover found. In these instances only a small number of foreign grains were present except in the case of one bee which had collected almost equal amounts of knapweed (*Centaurea nigra*) pollen and red clover pollen, the former having been collected first and a layer of red clover pollen added on the top. It would therefore appear that the honeybee when working red clover for pollen was usually very constant to this species on any one trip.

TABLE 3. *The number of bees in a group collected on the alighting board of the hive carrying full loads of red clover pollen and white clover pollen on* 25 *June* 1940

	a.m.					p.m.						
Hour during which bee carrying pollen returned to hive	7–8	8–9	9–10	10–11	11–12	12–1	1–2	2–3	3–4	4–5	5–6	6–7
Total no. of bees whose pollen loads were examined	9	23	24	54	73	62	81	65	72	72	58	46
No. of bees found to be carrying white clover pollen	2	7	8	14	28	38	57	48	23	25	18	11
No. of bees found to be carrying red clover pollen	0	3	5	22	21	10	6	9	30	33	21	8

CONDITIONS UNDER WHICH THE HONEYBEE WORKS RED CLOVER

As stated (p. 128) the honeybee visits red clover in search of either nectar in which case pollen is usually only collected incidentally, or in deliberate search of pollen. Honeybees searching for pollen only were found during 1939 and 1940, to visit red clover at all times of the day when the pollen of this plant was available to them, i.e. between approximately 9 a.m. and 8.30 p.m. G.M.T. Two peak periods of collection were usually noticeable, one about 10.30 a.m. and a higher peak about 3 p.m. According to Parker (1926) most of the leguminous plants, such as clovers, usually reach their peak of pollen dehiscence in the early afternoon, and that of white Dutch clover (*Trifolium repens*) appears to come somewhat earlier than that of red clover; thus competition between these species is reduced. In the early morning bees are frequently found working red clover for pollen; later in the day practically all the pollen gatherers entering the hive were found to be bringing in white clover pollen; and still later in the afternoon red clover pollen once more became the more abundant of the two (see Table 3).

The factors determining the visits of honeybees to red clover inflorescences in search of nectar and incidentally pollen, appear to be somewhat complicated. Scullen showed (Root, 1939) that the sugars in the nectar of a plant must reach a concentration of at least 17%

before honeybees collect it. Further, honeybees will desert one species of plant in order to work another as the abundance and concentration of their respective nectars varies. Generally speaking, provided the nectars of several species of plants abundant in a given district are all of approximately the same concentration, the greatest number of honeybees will be found working that species in which the nectar is most abundant and most easily obtainable. Almost throughout the summer of 1940 with its long dry periods the concentrations of the nectars of both red and white clover were practically identical, often as high as 60%, i.e. nearly saturated. Even so, it was frequently observed that numerous honeybees were working the white clover and few the red clover in search of nectar, despite the fact that the red clover was well within the flight range of the bees in the apiaries in which these experiments were conducted, and was probably more abundant than the white clover.[1] It has often been suggested that the corolla tube of first crop red clover is too long for the honeybee to be able to reach the nectar. Dunham (1939*a*), however, pointed out that the distance which a honeybee is able to reach for nectar in the corolla tube may be computed as approximately 7·9 mm., this figure being made up as follows: 6·3 mm. is the average length of tongue of the honeybee, 0·5 mm. the additional distance which a bee of the indicated tongue length can reach, and 1·1 mm. the average depth in the opening of the corolla tube into which a honeybee can thrust the anterior part of the head. The mean length of 500 corolla tubes collected at random in June 1940 was approximately 9·6 mm. as against a figure of 9·5 mm. for the experimental plants used by Dunham (1939*a*).

The honeybee can reach the nectar if it rises to 1·7 mm. or more. During the dry summer of 1940 this appeared to happen about once in every 4–5 days usually after a night of heavy dew when the honeybees were found working this plant for nectar. A heavy dew leads to the absorption of water by the nectar and consequently to a rise in height in the corolla tube, which may be sufficient to bring it within reach of the honeybee. A number (127) of measurements were made of the height to which the nectar in the corolla tubes rose, and it was found to vary between approximately 0 and 3·4 mm.

A moderately dry summer with dew at night is more likely to produce conditions under which the honeybee can work red clover for nectar, whereas a wet summer probably causes the plants to grow longer corolla tubes and the nectar to become more dilute.

Relative abundance of honeybees and bumble-bees on red clover

Counts were made at frequent intervals of the number of honeybees and bumble-bees working red clover on a strip 100 × 1 yd. along one side of the field. About 10 min. were taken in walking along the strip making the count. The results obtained during three days in June 1940 when little or no nectar was present in the flowers, and during three days in June when abundant nectar of a high concentration was present are shown respectively in Figs. 3 and 4. When nectar was absent the curve of honeybee activity shows the typical two peaks of pollen collecting activity, but when nectar was present the whole of the days' activity was much greater and the second of the days' peaks is considerably higher.

[1] Analyses of the total sugars present in the nectar samples were made by means of a modification of the Tillmans-Phillipi method (Pirie, 1936) using a Lovibond Tintometer. The amount of nectar required for each test was about 0·5 ml. and was collected by means of a fine capillary pipette thrust into a number of individual flowers. Ten samples were examined at each time and the mean of the results taken.

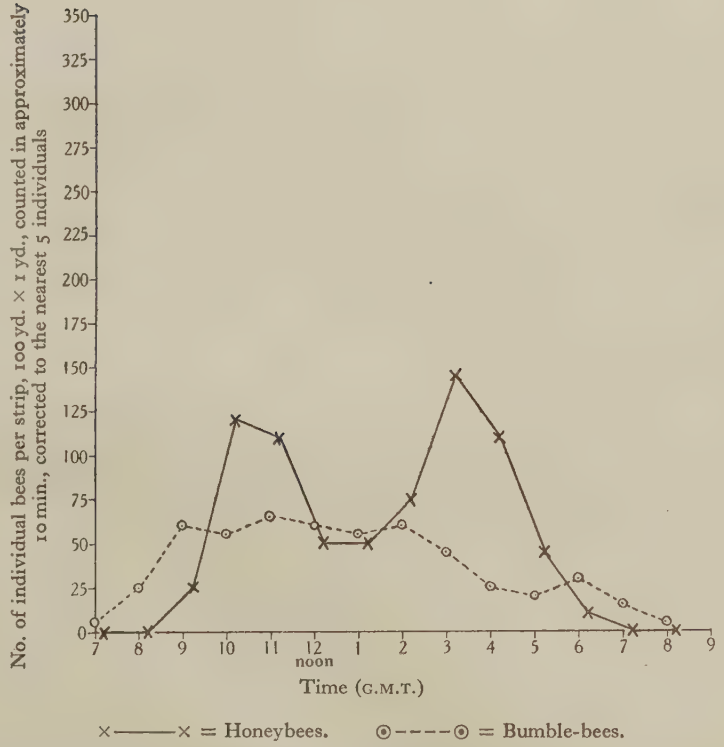


Fig. 3. Mean no. of honeybees and bumble-bees counted on a strip of red clover field, 100 yd. × 1 yd., in approximately 10 min. on 3 days when little or no nectar was present in the flowers in June 1940.

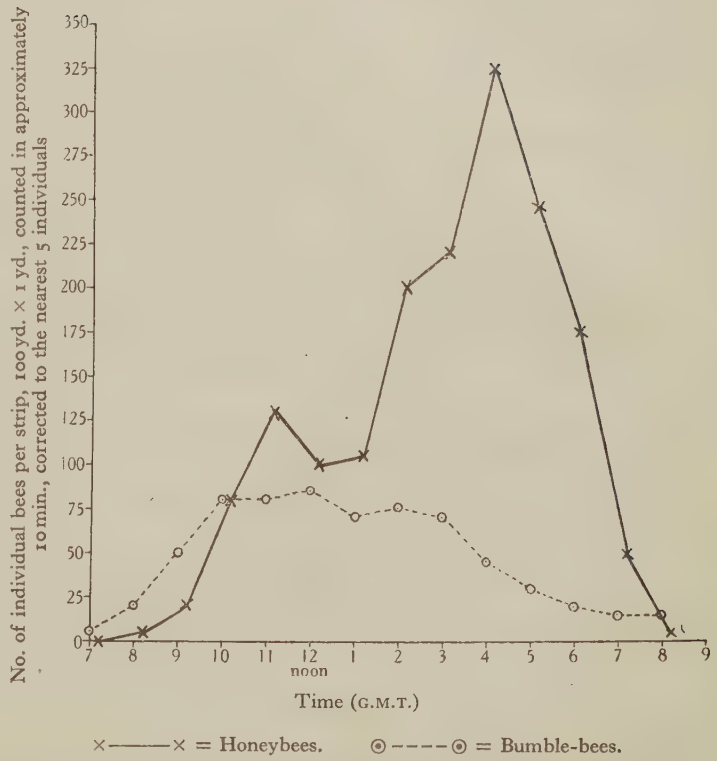


Fig. 4. Mean no. of honeybees and bumble-bees counted on a strip of red clover field, 100 yd. × 1 yd., in approximately 10 min. on 3 days when the nectar concentration was very high and the nectar over 2·5 mm. high in the flower tubes in June 1940.

Dunham (1939*a*) in Ohio found that about thirty species of insects are concerned with ed clover pollination; that in each year the honeybee was a major factor; and that, with the xception of the bumble-bee, the amount of pollination activity accomplished by other nsects was of little value. Although no accurate counts were made general observations trongly suggest that the same is true in this country. Dunham (1939*a*) also measured the elative rate of pollinating activity of six species of bumble-bees and honeybees, and found h-t with bumble-bees it varied from 16 to 37 flowers per min. and with honeybees from 6 to 12·5 flowers per min. Having information on the relative abundance of insects in a en area for each hour of the day for a three-year period, and also their rates of 'pollinating ivity', he found it possible to calculate in quantitative terms the relative percentage of lination performed by honeybees and bumble-bees respectively. Over this three-year riod, honeybees could be credited with having performed 82% of the cross pollination l bumble-bees with approximately 15%. The remaining 3% was carried out by other nenopterous, lepidopterous and dipterous pollinating insects. No comparable data lected in this country are available for making such a calculation; nevertheless since such ge numbers of honeybees were found to be present in the clover field at certain times, it uld appear likely that the honeybees were responsible for an important part of the lination of the seed crop.

Summary

Red clover pollen is available from about 9 a.m. to 8.30 p.m. (G.M.T.) in this country.

In the Harpenden district during 1939 and 1940 the honeybee worked first and second op red clover for both nectar and pollen. Of 484 honeybees working red clover whose ads' were examined in detail, 60% were nectar gatherers and 40% pollen gatherers.

The honeybee carries on the average approximately 284,000 red clover pollen grains per ad and visits a minimum of 284 flowers in collecting this load. The average time that cked honeybees took when collecting red clover pollen was 24·2 min., which is in good eement with the calculated figure.

Bees working red clover were remarkably constant to this species on any one trip; only in ..en cases were other grains found.

The honeybee exhibits two peak periods of activity when working red clover for pollen, ut 10.30 a.m. and 3 p.m. (G.M.T.). Probably the plant reaches its peaks of pollen iscence at these times. The honeybee probably collects pollen from red clover whenever is available in quantity.

The visits of honeybees to red clover to collect nectar and pollen are chiefly determined the height of the nectar in the corolla tubes.

When the nectar concentration was lower than that of other honey plants, or the nectar high enough in the corolla tube, bumble-bees were usually more abundant on red clover n honeybees, except at the peak periods of pollen collection. When conditions were right nectar collection five or six times as many honeybees as bumble-bees were often present.

Few insects other than honeybees and bumble-bees were found working red clover.

Dunham (1939*a*) found that honeybees performed approximately 82% of the cross-pollination of red clover, bumble-bees 15% and the hymenopterous, lepidopterous and dipterous pollinating insects 3%. No comparable data collected in this country are

available, but observations indicate that honeybees are responsible, for an important part in the cross-pollination of the seed crop.

I wish to take this opportunity of thanking Dr C. B. Williams and my colleagues in the Bee Research Laboratory for their interest and help in the investigation.

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THE SPREAD OF EELWORM IN COMMERCIAL NARCISSUS PLANTINGS

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(With Plate 7 and 1 Text-figure)

The stem and bulb eelworm (*Anguillulina dipsaci* Kühn) has become so widely distributed wherever narcissi are grown that a knowledge of the conditions which favour or restrict its spread is of great practical value to the commercial grower. It has for this reason been made the subject of special study by the Department of Plant Pathology at Seale-Hayne College by means of experiments in the College plots and by large scale observations and inspections on growers' farms.

Introduction of eelworm

Eelworm is introduced through the planting of infected bulbs: no evidence has been found of the pest being transferred from weeds or other susceptible hosts on to the daffodil crop in the field, although this has been shown to be possible experimentally (Hodson, 1931). In most cases eelworm has originated through bulbs having been planted without hot-water treatment, but there are many well-attested cases where eelworm has shown itself after inadequate hot-water treatment.

The number of bulbs affected with eelworm may be very small, and in the first year after planting they are difficult to find without very careful search. For example, in one field $1\frac{3}{4}$ acres in extent in west Cornwall early in 1939, a careful examination revealed only seven affected plants. The bulbs, var. King Alfred, had been planted the previous summer without hot-water treatment as no eelworm had been noticed before lifting. Such a small proportion of infected bulbs (7 in about 120,000 or 0·006 %) would very easily be missed even in quite careful inspections. The same stock in another field showed 33 infections in about 160,000 bulbs or 0·02 %. These figures show how difficult it is to be certain that large stocks are quite free from eelworm and prove the advisability of routine hot-water treatment.

Even in small plots it is very easy to miss small amounts of eelworm. In 1938 when careful regular inspections were made, a plot of Helios consisting of $5\frac{1}{2}$ beds about 20 yd. long was inspected on 18 Feb., 11 Mar. and 2 May. At the first inspection one severely affected plant and one plant with spickels were found. They were both missed at the second inspection, but on going over the beds again after ascertaining from the notebook the position of the infected plants, the badly infected plant was found, but not the slightly infected plant, even after prolonged search. At the third inspection no trace of eelworm could be found, the badly attacked plant probably having rotted away. The abundance of foliage growth at this late date makes inspection very difficult.

Spread of eelworm

Spread of eelworm is possible by: (*a*) eelworm carried in surface drainage water; (*b*) eelworm carried in soil attached to tools, boots, etc.; (*c*) dried eelworm in dead leaves harrowed or blown about over the fields.

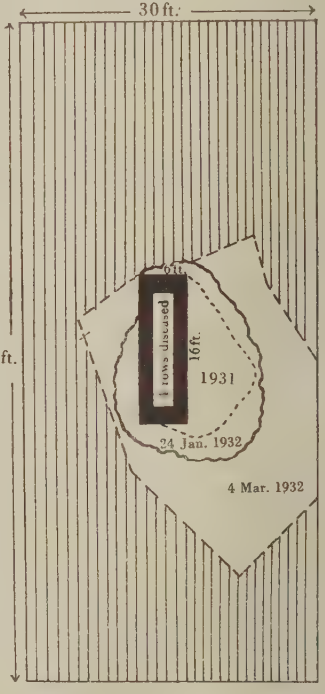
(*a*) The first is probably the most common method of spread particularly in south-west England, where the rainfall is high and the fields nearly always on a slope (Hodson, 1932). Its importance in U.S.A. was stressed by Stillinger (1934).

In 1930, an experiment[1] was laid down to study this method of spread in detail. A stock of 3000 bulbs of Emperor was planted after hot-water treatment in soil that had not grown daffodils before. The plot consisted of 24 rows, and in the centre of each of the four middle rows (rows 11–14) about 20 eelworm-affected bulbs were planted. On 17 March 1931, the incidence of eelworm in the plot was carefully observed. The newly affected plants were 1 in row 13 and 2 in row 14 below the eelworm plants and 7 in row 15 and 6 in row 16 alongside. The eelworm had spread, not down the row, but more to the east, actually in the direction of maximum slope of the plot. It may be concluded therefore that it had been carried chiefly by surface drainage. No further infections were noticed in 1931 but on 24 Jan. 1932 there was a little eelworm in rows 10–20 and on 4 Mar. 1932 the numbers of eelworm plants were as follows:

Row	Eelworm plants	Row	Eelworm plants	Row	Eelworm plants
7	1	13	7	19	37
8	4	14	10	20	27
9	3	15	29	21	21
10	12	16	21	22	18
11	5	17	34	23	12
12	10	18	37	24	8

The distribution is shown in Text-fig. 1 and Pl. 7, fig. 1. In every case the eelworm plants were below the original area of infection except an occasional plant in rows 14, 15, 17, 18, 21. These had probably been carried up slightly by cultivations.

There was only a slight increase on 8 May 1932. In 1933 the eelworm area was very much larger, but the character of the extension was similar to that of 1932, i.e. in the direction of greatest slope. Most of the bulbs affected in 1932 had rotted completely leaving a large gap (Pl. 7, fig. 2). It will be noticed that rows 1–10 on the western side were hardly affected by eelworm.



Text-fig. 1.

The losses in the flower crop due to eelworm also give a good picture of the distribution. The total crops in the years 1931–3 were 2047, 5119 and 6490 respectively, indicating a falling off in the third year. That this was due to eelworm is shown by a comparison between A, the unaffected area (rows 1–7), B, the moderately affected area (rows 8–14) and C, the badly affected area (rows 15–24). The average flower crop per row in each section was as follows:

	1931	1932	1933
A	89	236	372
B	72	188	233
C	94	215	224

The increase in the flower crop in the third year was very small in the affected sections compared with A. This has been the common experience of flower growers who have planted stocks affected with a small amount of eelworm, and have failed to notice their losses till the third season. The loss in bulb crop is still more serious. The average weight of bulbs lifted per row in the healthy section, A, was 30 lb., but in B and C it was 19 and 15½ lb. respectively.

The regular inspections carried out on many daffodil farms in Devon and Cornwall in 1937 gave many good examples of spread of eelworm in surface water, but in 1938 and 1939 when the inspections were repeated no such spread was observed. This is explained by the

[1] The experiment was started by W. E. H. Hodson who was Advisory Entomologist at Seale-Hayne Agricultural College before transferring to Reading University in 1931.

high rainfall in 1937 producing much surface water, whereas in the later seasons the soil was generally dry.

Rainfall (in.) at Seale-Hayne College

	1937	1938	1939
Jan.	7·37	5·59	6·95
Feb.	7·39	1·30	1·90
Mar.	6·01	0·09	1·65
Apr.	3·61	0·08	3·47
May	2·98	2·72	0·50

High rainfall in February and March means much surface water when all the leaves are well through the ground, and therefore maximum spread of eelworm. In the 1937 inspections in February, March and early May there was a very large increase in eelworm between the first and second inspections, and a moderate increase between the second and third. As a result of this exceptionally favourable spring many fields which showed very small amounts of eelworm in 1937 showed very large amounts early in 1938. This was a great surprise to many growers, some of whom had failed to recognize the small amount of eelworm present in 1937 and wondered where such a bad attack of eelworm had originated. In 1938, however, there was no increase in eelworm after the first inspection and there was no further increase in 1939.

(*b*) It is difficult to demonstrate the transference of eelworm in soil attached to tools, boots, etc., as actually occurring in the field, and cases which have been observed as apparently due to this have followed hot-water treatment with baths which have been proved by direct tests to be inefficient.

A probable way in which eelworm spreads is in the soil on workers' boots, particularly at flower picking. Leaves as well as soil become attached to the workers' boots and may move up and down the rows greatly helping the spread in wet weather. Eelworm might conceivably be spread in the juice that exudes on to the pickers' hands, but as the eelworm is generally confined to spickels and more often on the leaves than on the stems, this does not seem likely to play a very large part. It may be more important in the case of Polyanthus Narcissi in which eelworms often fill the flower stalk in great numbers.

(*c*) The spread of eelworm in dried leaves is far more prevalent and serious than is generally realized. An example of its importance is provided by a field in west Cornwall which was systematically inspected in 1937. No eelworm was found on $\frac{3}{4}$ acre of Horace on 11 Feb. and 4 Mar., but on 29 Apr. one affected plant was found. This was carefully lifted together with adjacent plants. Evidently one bulb of many thousand treated had escaped the hot-water treatment. It was expected that all the infection had been removed, but when the field was inspected on 18 Feb. 1938 an enormous amount of eelworm was found, only 15 beds out of 50 being free. This heavy infection could not have come from the small amount that escaped hot-water treatment. The explanation was that in the variety King Alfred in another part of the field there were one or two badly affected patches in 1937 which were not lifted, and later in the season the field was cross-harrowed to kill the weeds. This would spread the eelworm all over the field, and explains the abundance of infection in the Horace and in other varieties in the same field.

These observations also illustrate the important practical point that when any part of a field shows eelworm infection, the whole field must be regarded as an infected field, and all the bulbs lifted from that field must be hot-water treated in order to make sure of destroying

the eelworm. A similar conclusion in respect of eelworm in sugar beet was reached by Petherbridge (1938), who states that if one of several smallholdings in one field becomes infected the whole field should be treated as an infected field.

The above observations explain the common opinion that eelworm becomes serious more quickly in commercial plantings than in gardens or wild plantings. The spread of eelworm in gardens is hindered by footpaths and grassy banks and amongst bulbs naturalized in grass there is practically no spread, there being no cultivation and not much surface drainage. Eelworm affected bulbs die out but the unaffected majority remain free from the pest.

Dates of infections by bulb eelworm and persistence of small infections

It is important to know at what periods of the year infection by bulb eelworm can take place as this knowledge will have a practical bearing on the steps taken to reduce bulb infection. Infection takes place in the foliage or in the tissues of the neck of the bulb, but never directly into the bulb or roots (Hodson, 1932). In the field according to our observations, infection of the neck of the bulb without leaf infections is rare, although it probably takes place more readily in pots. From the practical point of view, therefore, the important question is, during what period is infection possible into the leaves? Spickels have been observed as early as January so that infection probably takes place in most varieties from December onwards. In early shooting varieties such as Sol d'Or infection can probably take place earlier. Spickels continue to appear on the later leaf growth right through the season and pot experiments in 1934–5 by one of us (L. N. S.) with the variety Golden Spur have shown that infection takes place readily any time from January to May. In a wet season very late infections are probably abundant and may account for the large increase in the amount of eelworm observed the following season. They may also explain the apparent missing of a season which might at first sight suggest that eelworms can remain dormant through a growing season. The following example will make this clear.

On 7 Sept. 1933 Emperor bulbs from the eelworm plot referred to before (p. 136), were hot-water treated for 2¼ hr. This would kill most of the eelworm but would probably let a few escape. The bulbs were planted in new ground containing no other daffodils and in 1934 no eelworm was observed: 19 Apr. was the last date of examination. In March 1935 spickels were observed on three plants. Eelworm was found in the leaves but the bulbs were clean. It is suggested that the explanation of this is that the very small amount of eelworm occurring in 1934 missed observation but that the infection entered the soil and caused leaf infections to appear in 1935.

Another example of this survival of minute amounts of eelworm is shown by one of the commercial holdings in south Devon inspected by us over a number of years. All the stocks were free from eelworm until 11 Feb. 1937 when it was noticed in a stock of Sunrise (newly purchased and planted without hot-water treatment). On 18 Mar. there was a patch 2 ft. square showing about 80% infection, and this was dug out a few days later and the whole bed was lifted and hot-water treated in a hot-water bath on another farm in the district. Regular inspections on the holding in 1938 and 1939, giving particular care in the examination of the Sunrise, showed no eelworm. The affected bed had been roped off so that no cultivation could be carried out to spread eelworm in the soil, neither was it possible for soil drainage to reach other bulbs.

The Sunrise bulbs were lifted again in the summer of 1939 and planted without hot-water treatment in soil that had not grown narcissi before. Inspection on 20 Mar. 1940 revealed the presence of eelworm in several plants (12 in 14,000). Live eelworms were found in the leaves in all these, but only in one plant were any found in the bulb. It was concluded from this that there must have been a very small amount of eelworm carried over in the soil attached to the bulb or in the bulb scales in the neck. It was not likely there was any unsuspected soil infection, as other varieties alongside were free. This conclusion was confirmed by a careful examination of the plants which had escaped lifting in 1939.

A number of slight swellings were found, but microscopic examination showed they did not contain eelworm. In the course of this examination a very faint spot was noticed and found to contain a live bulb eelworm; presumably this was the early stage of a spickel. This scarcity of eelworm showed how small must have been the number that managed to carry on the infection in the stock during 1938 and 1939 without being noticed.

Can eelworm be eradicated from a bed by cutting off infected leaves?

In beds exposed to eelworm infection by surface drainage, only the leaves are at first infected, and theoretically it should be possible to prevent the bulbs from becoming infected by cutting off at frequent intervals all the leaves showing spickels.

On 30 Mar. 1938 plants of Sunrise from which all the leaves has been cut off were transplanted to pots of clean soil and observed next year to see if they would grow up again free from eelworm. The growth was rather weak and two of the plants showed eelworm in the bulb. Sunrise is a susceptible variety, and the cutting off of all the leaves and the transplanting would cause severe injury to the plants. Such drastic treatment is not to be recommended, but the regular removal of infected leaves from plants in the beds and the lifting of the bulbs at the normal time is more practicable. This was done in the plots at Seale-Hayne College in small stocks of the varieties Gloria Mundi, Cynosure and Croesus, and it was noted that no bulbs were lost at harvest time. This is only the case, however, on a small scale or in a dry season and when the eelworm infection is not too severe. Where the spickels are very numerous, or if some distortion and yellowing are present, there is certain to be loss of bulbs from the eelworm, and it is better to dig up such severely affected plants as soon as observed.

In rare cases, very small eelworm infections may fail to establish themselves. This was noted in one stock of Double White which was regularly inspected. Four plants showing spickels were found in one corner on 16 Mar. 1937 and were found again on 3 May and one additional plant. No trace of eelworm was seen in 1938 and 1939 in spite of prolonged search. The variety Double White appears late and the soil is usually dry when it makes most rapid growth. It is probably for this reason that Double White is so often free from eelworm in the south-west.

On the large commercial scale it is better to dig up the whole patch, where eelworm has been observed, not merely the odd infected plants. This was done in a number of instances in 1938 and 1939, and it was noted that there was little or no spread in the following seasons. There is a danger of considerable spread, however, in wet seasons such as that of 1937, and it is better to lift the remainder at the end of the season. This is not always commercially practicable, and then it is recommended that a trench be dug around the lifted patch as this will prevent the spread of surface water and also help to prevent the passage of workers or cultivators over the infected patch.

If sufficient care is taken it is theoretically possible to eradicate eelworm completely by these methods, but no instance where this has been accomplished has been found in our observations.

How long a period must elapse before bulbs can be planted in an infected field?

This is an important practical point as the acreage of the average bulb grower is strictly limited so that long rotations are not always possible. Observations of van Slogteren (1923) suggest that eelworm cannot live in soil in the open free from bulbs for longer than one year, although in a dry state they can remain alive for several years (Goodey, 1936). There is now a large amount of practical experience that suggests that daffodils can be replanted after a three years' interval, but it is not certain that eelworm would be completely eradicated in such a time under all conditions. At Seale-Hayne College a plot showing severe eelworm attack in the spring of 1936 and lifted in that summer, except for a small bed of Double White not lifted till August 1937, was replanted in September 1939 and showed no trace of eelworm in 1940 but showed a few infected plants early in 1941. In commercial practice, a great many bulbs are unavoidably left behind at lifting time and they should be dug up the following spring. We have rarely observed eelworm in such volunteers, however, and it is probable that most of the infected bulbs not lifted, rot away during the summer, and the survivors being scattered do not readily become infected. If re-infection did occur, however, the beneficial effect of the three years' rest period would be nullified.

Practical conclusions on the value of field inspections

Regular inspections accompanied by the digging out of infected plants are of inestimable value to the grower and are necessary to prevent the occurrence of serious losses in his bulb stocks. The date of the first inspection will depend on the variety and the district, but in most cases may be taken as some time in February. The absence of eelworm at this date must not be taken as proving that the pest does not occur, as badly affected plants are late in coming through. Another inspection must be carried out about a month later. Later inspections are difficult owing to the abundance of foliage. From our experience we estimate that two men, who are used to the work, can inspect and rogue 1 acre in 1–2 hr., unless the amount to be dug up is very considerable. It should be noted that the most efficient inspections are carried out early in the season before flowering. This is of great assistance to the grower in planning his labour requirements.

The next question arising from this work is: Is it practicable to give certificates to growers as to the freedom of their stocks from eelworm? This would be very valuable to the purchaser, as it would save the necessity of hot-water treatment; but to be of any value it must be absolutely reliable, and our experience has shown that no guarantee can be made. The only exceptions possible are with farms that have been regularly inspected and found to be *entirely* free from eelworm for a number of years. The expense of such repeated thorough inspections by outside officials would be prohibitive. The rule still holds, therefore, that newly purchased bulbs should always be subjected to correct hot-water treatment, whatever their source.

Summary

Eelworm is introduced in commercial narcissus plantings by stocks of bulbs containing a small percentage infected with eelworm, the stocks having received no hot-water treatment or only inefficient treatment. Spread of eelworm is chiefly in the surface water and takes



Fig. 1.



Fig. 2.

BEAUMONT AND STANILAND—THE SPREAD OF EELWORM IN COMMERCIAL NARCISSUS PLANTINGS (pp. 135–41)

place mostly in the direction of the surface drainage. Soil attached to tools and boots may be a subsidiary means of spread, but probably less important than the movement of dried leaves containing eelworm, especially that brought about by cross-harrowing. Narcissus leaves can become infected any time from January to May. Late infections are easily overlooked and lead to surprising increases in the amount of eelworm in favourable weather. In dry weather small amounts can persist, without being easily observed, over one or more seasons. Eelworm infection can be reduced by cutting off leaves bearing spickels, but the method can be recommended only in small gardens. In commercial plantings, affected patches should be dug out completely and surrounded by a trench to prevent eelworm spread. Infected fields cannot safely be replanted for at least three years after the bulbs have been lifted, and volunteer plants must have been promptly removed. Field inspections are of great value to the grower, but it is not considered practicable for outside authorities to award certificates based on the results of inspections, guaranteeing freedom of bulb stocks from eelworm.

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EXPLANATION OF PLATE 7

Fig. 1. Plot of narcissus, var. Emperor, at Seale-Hayne College in the spring of 1932. The bed is in its second season and the photograph shows the spread of eelworm from the four infected rows in the middle bounded by white pegs.

Fig. 2. The above plot in the spring of 1933 showing still greater spread of eelworm.

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A LABORATORY SPRAYING APPARATUS AND TECHNIQUE FOR INVESTIGATING THE ACTION OF CONTACT INSECTICIDES. WITH SOME NOTES ON SUITABLE TEST INSECTS

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(With Plate 8 and 5 Text-figures)

To ascertain whether any substance is likely to be useful as a contact insecticide two separate investigations have to be made: a laboratory examination of the effect of the substance on insects, and a study of it in the field under the conditions in which it is to be used in practice. In order to ascertain how a contact insecticide can be applied to the best advantage, it is necessary to make a detailed study of its effect both qualitatively and quantitatively, and this can be most satisfactorily carried out under the controlled conditions only to be obtained in the laboratory.

Laboratory studies on contact insecticides in a fluid medium can be classified under three heads: (1) The discovery of new substances which possess insecticidal activity, and a general investigation into their possibilities. (2) The detailed study of any given substance to determine its physiological action and its effect on particular species of insects. (3) The study of the effect of varying the medium in which the toxic substance is applied, of the methods of application and of factors extraneous to the toxic substance.

The first requisite for the study of these three problems is a method of applying the toxic liquids in equal doses to large numbers of individual insects of a wide variety of species. The method should also be such that the individual doses administered may range from small to large. The second requisite is to have available a wide range of species with representatives from all the important orders of insects. It should be possible to rear some, at any rate, of these species so that large numbers are continuously available; for this purpose, species with a short life cycle and no seasonal rhythm are most suitable.

In the present work most of the time was devoted to the method of applying the dose of insecticide, but some preliminary work was also done on the selection and rearing of suitable test insects. Once a suitable method of application of the insecticide was found, the extent to which results obtained with any given species and instar are generally applicable, depends on the degree to which specificity of effect occurs, and this will probably vary both with the insecticide and the insect. The information on this subject is scanty, and it is desirable that more data should be gained on the subject of specificity as a preliminary to the selection of a range of species as test insects. In the meantime it has to be assumed that an insecticide which fails to kill the adult of one or two species of insect which have not so far been found highly resistant, is of little value as an insecticide.

Apparatus and methods at present available

Tattersfield (1939) reviewed biological methods of testing insecticides, including a brief description and discussion of the laboratory methods of testing liquid contact insecticides so far used. The means of applying liquids to the integument of an insect can be divided into

three classes: (1) spraying, (2) dipping, (3) the use of the micropipette. The dipping and micropipette methods were discarded because they were too far removed from practice and could give no data on some of the problems outlined in the preceding paragraphs. The dipping method was thought to have the further disadvantage that stomach poison and a contact poison effect might be indistinguishable. The micropipette method becomes extremely laborious if the large number of insects required for the accumulation of data suitable for accurate statistical analysis are used.

The desirable attributes of a laboratory spraying apparatus are: (1) that a given quantity can repeatedly be deposited on the sprayed area, so that equal doses may be applied for purposes of comparison; (2) that the spray should be evenly deposited over the sprayed surface so that insects distributed over that area may each receive an equal dose; (3) that a sufficiently large area should be evenly coated, so that insects of various sizes and rates of locomotion may be used; (4) that it should be possible to vary the amount of spray deposited within wide limits from a very light deposit to a very heavy deposit; this is most necessary where media which are themselves toxic are used; (5) that the apparatus should be suitable for use with a wide variety of fluids.

Omitting the lethal chamber methods employed for testing fly sprays, the spraying methods that have been used can be put under four heads: (1) the Tattersfield method; (2) the Campbell turn-table method; (3) the method of O'Kane *et al.*; (4) the method of Hartzell & Wilcoxon.

The Tattersfield method (Tattersfield & Morris, 1924; Tattersfield, 1934) has been successfully used for the qualitative and quantitative evaluation of a large number of insecticidal substances and is the only spraying apparatus for which full data of its physical and biological performance have been published. It has the advantage of simplicity and accuracy. Its disadvantages are that the area that can be relatively evenly coated with spray is small so that only small slow-moving insects or the inactive stages of insects may be used, and it is difficult to reduce the weight of deposits beyond a certain minimum value.

The Campbell turn-table method (Jones *et al.* 1935; Campbell & Sullivan, 1938) appears to have been widely used, but there are very few data on its performance, both physical and biological. On a priori grounds, it appears likely that the spray is evenly deposited over the sprayed area, but the total amount deposited after each spraying may not be constant, and it also appears that it would be difficult if not impossible to apply other than light deposits. Further, the insects must be rendered inactive before treatment, since they necessarily have a long period of exposure, during which they might crawl off the sprayed area if not previously inactivated.

The method of O'Kane *et al.* (1930) does not appear to be sufficiently flexible and quantitative. The method of Hartzell & Wilcoxon (1932) does not appear to fall into the category of quantitative laboratory testing; it has more the nature of an accurate preliminary field trial, and presumably was not designed to answer the questions that are raised here.

Evolution of apparatus. Sources of error in replication and distribution of deposit

When it became necessary to devise a laboratory spraying apparatus suitable for testing contact insecticides on a wide range of species of insects, the Tattersfield apparatus was taken as a starting point, since this was the only apparatus the performance of which had been fully investigated and published. The outstanding disadvantage of this apparatus was

the small area that could be covered without a severe falling off of the amount of spray deposited.

By using a variety of atomizing nozzles to produce the jet of spray it was found that all of these, when applied directly to a surface, give a very much heavier deposit in the centre falling off rapidly to the outside of the sprayed area. This difficulty could be overcome by breaking up the cone of spray after it issued from the atomizing nozzle by inducing turbulence, by spraying through a tube. It was found that a number of factors influences the degrees of turbulence and thus the evenness of the deposit, the most important being: (1) the diameter of the tube; for tubes of a given length the smaller the diameter the greater the turbulence: (2) the length of the tube; the length of tube necessary to obtain sufficient turbulence to produce an even deposit varied inversely with the diameter of the tube: (3) the nature of the apparatus at the top and bottom; closing the openings at the top and bottom of the tube both tended to increase turbulence and produce a more even deposit: (4) volume and rate of flow of air; increased volume and rate of flow of air produced by raising the air pressure cause greater turbulence under given conditions.

With these facts in mind an apparatus was constructed as shown in the photograph (Pl. 8 *a*). The apparatus consisted of a square tube of glass and metal, the upper narrower part of the tube acting as a mixing tower in which turbulence took place and a lower wider part acting as a settling chamber in which the liquid droplets settled out on the spray plate. An aerograph M.P. paint gun was used to atomize the spray fluid. This instrument was closed at the bottom when spraying was taking place and was closed at the top except for four small exit holes to relieve the pressure. It was constructed at the Imperial College of Science and Technology and used there for a number of experiments which have since been published (Potter, 1938; Potter & Musgrave, 1940; Callaway & Musgrave, 1940). This was used with insecticides in an oil medium alone; the apparatus was found to give a fairly even deposit over a 6 in. plate, but the total deposit varied considerably from one spraying to the next, and throughout the work the total deposit had to be checked by weighing. It was further necessary to atomize large amounts of material in order to obtain sufficient deposit on the spray plate, a very high proportion of material being thrown out on to the sides of the spray tower. At this point the work was taken up at Rothamsted where more attention was given to aqueous fluids than to sprays with an oil base.

Using the type of apparatus shown in Pl. 8 *a* and *b* the variation in total deposit in a series of spraying trials was normally from 10 to 20% and might be as high as 30%. The causes for this variation were obscure and a large number of experiments were made to elucidate the difficulty, numerous different atomizing nozzles and a variety of lengths and shapes of tube which were closed both top and bottom in varying degrees being used. A short series of experiments was also made on what were considered to be the most likely causes of variation, i.e. (1) effect of air currents, (2) effect of temperature and humidity, (3) electrostatic effects.

(1) *Effect of air currents.*

Although an even distribution can be made by inducing turbulence, it seems as if this is also liable to introduce errors in the replication of the amount of deposit from one spraying to the next. A possible reason for this is that the critical velocity at which an air stream becomes turbulent is not constant even under relatively constant conditions. It is therefore

probable that turbulence commences at different depths in the spraying tower in different sprayings, and since the higher up in the spray tower turbulence starts, the more material is thrown out on the sides of the vessel and the less reaches the bottom, it can easily be understood that this instability of the point at which turbulence commences is a likely source of error. No means have been found of verifying this experimentally, but all the general evidence gathered from over 2500 measurements by weighing of deposits points to the fact that where there is a great deal of turbulence there is a greater liability of error.

It appears that evenness of air pressure over the sprayed area is one essential in the production of an even deposit. An apparatus was set up to produce an even deposit and then a separate jet of air alone was directed on the plate upon which the deposit was falling; at whatever point the air jet impinged on the plate a very heavy deposit was formed.

When these points had been established, all efforts were directed to evening out the air pressure over the sprayed area with the minimum of turbulence. This meant, among other things, working at considerably lower air pressures than had previously been used. It was also found that an adjustable gap at the bottom of the spray tower was a convenient method of regulating the air currents. This was obtained by having a circular plate of the same area as the bottom of the tower mounted in such a way that the gap between it and the spray tower could be varied and at the same time accurately adjusted. As the gap was increased, so the amount of turbulence decreased and the deposit tended to become heavier at the centre. By adjusting the gap to the maximum which could be used to produce an even deposit, the latter can be obtained with the minimum of turbulence.

(2) *Effect of temperature and humidity.*

Using aqueous sprays, little difference could be detected for temperature changes of 1–2° C. Differences above 5° C. appear to affect the deposit, probably due to the alteration produced in the saturation deficiency.

With the large surface area produced by atomization, evaporation effects must be important. That this is so is shown by using a volatile liquid such as alcohol or acetone when only a very small proportion of the material reaches the spray plate, but it does not appear that a change of 5 % in the relative humidity has any detectable effect when water is used as the medium at normal laboratory temperatures. No experiments were made to determine the separate effects of humidity and temperature, and it frequently happens that a large change in the relative humidity is accompanied by a considerable change of temperature, so that these effects must be considered together. Small changes in either do not appear to be important.

(3) *Effect of electrostatic change.*

It is known that when liquids are atomized the particles may acquire an electric change. The magnitude and sign of this charge varies with the liquid and the conditions. This matter as it affects spray deposit has been studied by Wampler & Hoskins (1939). Preliminary experiments were made to find out if this charging of the atomized particles was likely to be a cause of serious variations in deposit under the experimental conditions described.

First experiments were done by spraying liquids on to a metal target and conducting the charge off the target either to a gold-leaf electroscope or to a valve electrometer. Distilled water was first used and a much smaller charge was produced when the target was sprayed

through a glass spray tower than when it was sprayed directly. This was almost certainly due to the deposition on the sides of the tower, much less liquid reaching the target when it is sprayed through the tower. It was then found that drops of liquid dripping from the glass tower were highly charged, each drop causing a violent deflexion on the valve electrometer. When distilled water was sprayed through an earthed metal tower, the particles reaching the target were still charged, but the drops from the tower were not charged. When water containing traces of saponin was sprayed the charge was doubled and a very large charge was obtained with a very dilute emulsion of xylol in water. Water containing a little dissolved electrolyte gave no detectable charge.

It was clear from these experiments that, at least under certain conditions, electric charges might be a source of variation; more particularly, it appeared that a glass tower was liable to become charged and provide an electric field of varying intensity through which the atomized droplets had to pass. It was therefore considered that it would be preferable to use an earthed metal tower which would not only not become charged itself, but would provide a faraday screen, thus giving a uniform field through which the particles passed. When an earthed metal tower was used, errors of replication were sensibly reduced. This cannot with certainty be ascribed to a reduction of electrical effects, since the metal tower used, although similar was not exactly the same shape as the glass one which it replaced; it is, however, very likely that the slight difference in shape was immaterial, since towers of various shapes had previously been tried without greatly affecting the errors.

Further investigation into electrostatic effects was not made, since to do so would mean a long and involved study, which although of value might not be directly applicable to the work in hand. The work on electric charge was only carried out after a considerable amount of experimental work on the apparatus had already been done, the instrument shown in the photograph (Pl. 8 *b*) being used for a large amount of insecticide testing. This apparatus gave a good distribution, and errors of replication of the deposit were usually within 15%. The apparatus finally decided upon as being the most suitable is described in the next paragraph.

Construction of final apparatus

This consists of a reservoir and a specially designed atomizing nozzle mounted on a small circular plate carried on three bars, each at an angle of 120° with the other. The end of each bar rests on the top of the metal tower through which the spray is directed on to the spray plate or dish. The spray plate or dish is carried on a table formed by the bottom plate which has a universal adjustment, the whole being mounted on a wooden stand. (See Pl. 8 *c*.)

The atomizing nozzle.

Numerous types of atomizing nozzles have been tried, including paint spray guns (Aerograph type M.P.), air brushes (Aerograph), water spray nozzles (Aerograph type F.N. with various sizes of nozzles and cap), specially constructed nozzles by F. W. Pellant, similar in type to those used on the Tattersfield-Morris apparatus, and a variety of scent-spray nozzles and nozzles constructed in the laboratory. It was found that all these nozzles were capable of fairly good replication of deposit when sprayed directly on to a surface so that at least 20% of the material in the reservoir was collected and no modification of the direct cone of spray was made, the difference between the highest and lowest readings ranging from 4 to 10%. Under these conditions, however, there was invariably a great falling off in amount of deposit from the centre to the outside. When any attempt was made to modify the cone of spray to produce a more even distribution, the errors in replication of deposit were considerably increased in all the nozzles, the difference between the highest and lowest deposits for a series being from 15 to 30%. The degree of atomization with most of the nozzles used was adjusted by means of a needle valve in the liquid feed; in practice this valve tended to produce clogging, thus markedly

altering the weight of deposit and taking up considerable time for adjustment. A further disadvantage in all the nozzles available is that the liquid jet is almost invariably not quite centrally placed in the air jet, and no means are available whereby the jet may be centred in order to distribute the liquid evenly over the cone of spray. Further, there are no means of adjusting vertically the position of the tip of the liquid jet in relation to the tip of the air jet, an adjustment that markedly affects the degree of atomization.

Accordingly an atomizing nozzle was constructed which is as simple as possible, but has the two adjustments last mentioned (see Pl. 8 *d*, and the scale diagram Text-fig. 1). It consists essentially of two parts: (*a*) a top circular brass plate (*TP*) 2 in. diam. and $\frac{1}{4}$ in. thick, on which is mounted a liquid jet (*LJ*) with an adjusting nut (*AN*) working against a friction washer (*FW*) for moving the jet up and down, the liquid feed tube (*LFT*) and the liquid reservoir (*R*); (*b*) a bottom circular brass plate (*BP*) 2 in. diam. and $\frac{3}{16}$ in. thick joined by a narrow brass neck to a mounting plate (*MP*) in which is bored the air jet (*AJ*). The tube to the compressed air supply (*AT*) is led into the brass neck.

When assembled, the top plate is screwed to the bottom plate by means of three adjusting screws (*AS*) and there is a compressible rubber washer (*W*) in between. The two plates, screws and washer together form a universal joint. The liquid feed tube must form an airtight joint in the top plate, but at the same time be capable of movement up and down; this is done by having a stuffing box (*SB*), through which the tube passes, mounted on the top plate.

In order to adjust the nozzle for working, the liquid jet is first roughly centralized by means of the three adjusting screws (*AS*); it is then pushed through the liquid jet as far as it will go and adjusted to the required depth by tightening the adjusting nut (*AN*); finally, it is accurately centred by means of the adjusting screws (*AS*). The following are the essential dimensions of the various parts: air-jet orifice, 0·0635 in.; liquid jet orifice, 0·0135 in.; internal diameter of compressed air tube $\frac{1}{16}$ in.; thickness of rubber washer, $\frac{5}{32}$ in.; internal diameter of liquid feed, $\frac{1}{16}$ in.; capacity of reservoir, 10 c.c.

This nozzle worked well in practice; the accurate centring of the liquid jet in the air jet helps to obtain a symmetrical and even distribution and the absence of any form of needle valve in the liquid feed reduces the risk of blocking. The normal function of the needle valve is to alter the degree of atomization, but this purpose can be achieved in the nozzle described by altering the air pressure and by adjusting the position of the tip of the liquid jet relative to the orifice of the air jet.

Atomizing nozzle mounting (Pl. 8 *d*).

The atomizing nozzle is mounted on a plate of the same diameter as the mounting plate on the nozzle, i.e. $2\frac{1}{8}$ in. This plate carries three bars extending out radially, each at an angle of 120° with the other. The bars are $3\frac{3}{8}$ in. long measured from the circumference of the plate, $\frac{1}{2}$ in. wide and $\frac{3}{16}$ in. thick. 2 in. from the end they carry adjusting screws which work against the inside of the spray tower, by means of which the nozzle is centred in the tower. $\frac{5}{16}$ in. from the end are adjusting screws which work against the top of the tower and by means of these, the cone of spray can finally be adjusted to spray down the centre of the tower.

The spray tower.

This consists of a metal tube which is earthed. In the apparatus described this is of galvanized iron $\frac{1}{32}$ in. thick, but it would be preferable if constructed in some form of thicker rustless material. The tube is 26 in. long, both the top and the bottom having a 1 in. flange. The internal diameter of the top is $6\frac{1}{4}$ in. tapering to $5\frac{3}{4}$ in. in 12 in., the rest of the tower being of uniform $5\frac{3}{4}$ in. bore. The flange at the top of the tower is fitted with three adjusting screws working against the top of the stand, by means of which the tower is levelled.

The spray table with universal adjustment (Pl. 8 *c* and Text-fig. 2).

In order that the correct deposition and distribution shall be maintained, the bottom of the tower must be covered by a plate adjusted so that it is centrally placed and at a given distance from it. Since this gap is too narrow to allow the insects to be placed in position at the bottom of the tower it must be possible to lower the plate, put the insects on it, and raise it again to the correct position quickly and easily. The following arrangement is used: The spray plate (*T*) on which the insects are placed, either in a dish or otherwise, is a metal circle of $6\frac{3}{4}$ in. diam., thus fitting exactly over the bottom of the spray tower. The spray plate carries four adjustable angle-brackets (*AB*) to hold the spray dish, and adjustments are present for centring the dish under the bottom of the tower. From the centre of the spray plate, a pillar (*P*) of $\frac{1}{4}$ in. brass rod runs through a hole bored in a solid metal cylinder ($\frac{5}{8}$ in. diam.)

which is itself fitted in a hollow tube (Tu_1) of the same diameter. The pillar can be adjusted to the requisite height in the solid metal tube then fixed with the Grub screw (g); by this means the gap between the bottom of the tower and the plate can be accurately adjusted. The tube Tu_1 is fitted into a second tube (Tu_2) $\frac{3}{4}$ in. ext. diam. in which it is a sliding fit. The tube Tu_2 fits inside a wide tube

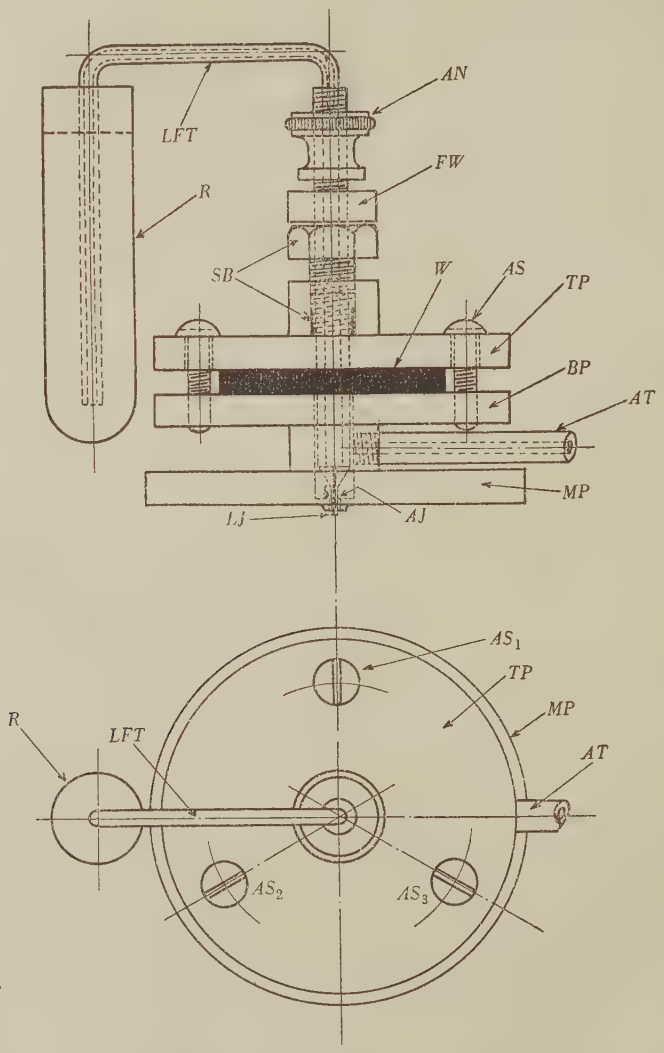


Fig. 1. Cross-section and plan of atomizing nozzle. Scale full size.

AJ, air jet; *AN*, adjusting nut; AS_1, AS_2, AS_3, adjusting screws; *AT*, air tube; *BP*, bottom plate; *FW*, friction washer; *LFT*, liquid feed tube; *LJ*, liquid jet; *MP*, mounting plate; *R*, reservoir; *SB*, stuffing box; *TP*, top plate; *W*, rubber washer.

or cylinder (Tu_3) $2\frac{1}{2}$ in. ext. diam. and 4 in. high and is held in position by six centring screws (S_1–S_6) arranged to project radially into Tu_3, three screws at the top and three screws at the bottom. The tube Tu_2 is prevented from falling through tube Tu_3 by the flange *FL*. The tube Tu_3 is mounted on a base plate (*BP*) which has a $\frac{1}{2}$ in. hole, to allow the passage of tube Tu_1 and Tu_2.

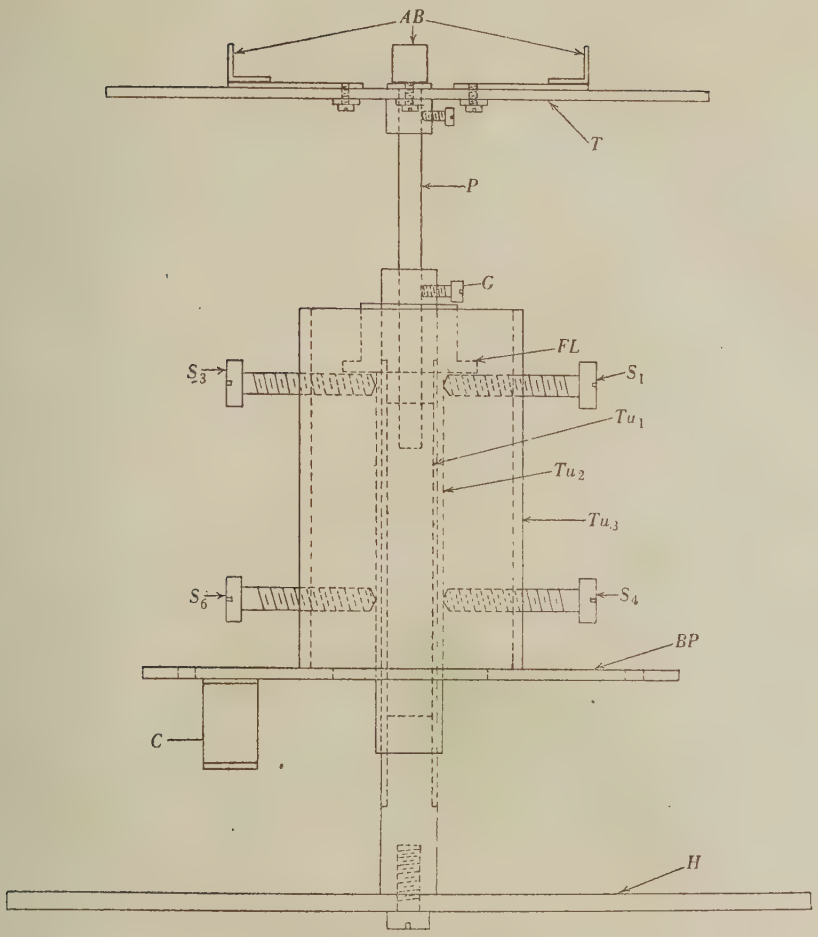


Fig. 2. Spray table with universal adjustment. Longitudinal section. Scale half-size.

AB, angle brackets; *BP*, base plate; *C*, catch; *FL*, flange on Tu_2; *G*, grub screw to hold pillar *P*; *H*, handle; *P*, pillar; S_1–S_6, centring and adjusting screws; *T*, spray table; Tu_1, inner sliding tube; Tu_2, outer fixed tube; Tu_3, cylinder to hold centring and adjusting screws.

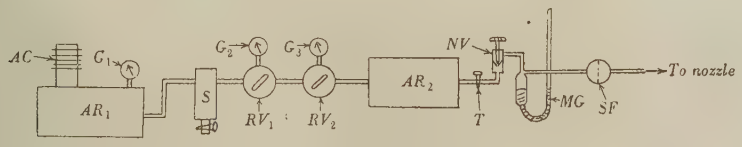


Fig. 3. Diagram of compressed air supply to nozzle, showing system of filters, pressure regulators and stabilizers.

AC, air compressor; AR_1, air receiver of air compressor; AR_2, small air receiver to equalize flow; G_1, first pressure gauge; G_2, second pressure gauge; G_3, third pressure gauge; *MG*, mercury pressure gauge; *NV*, needle valve regulator; RV_1, first reducing valve; RV_2, second reducing valve; *S*, oil separator and filter; *SF*, sintered glass filter; *T*, tap.

The base plate is fixed to struts on the stand holding the tower so that the spray table is as nearly as possible directly underneath the tower. The final centring of the spray plate beneath the tower is done by means of the screws S_1–S_6. In spraying practice, the tube Tu_1 is allowed to slide down to its full extent in tube Tu_2, leaving a wide gap between the bottom of the tower and the plate. The insects are put on the plate and the latter then raised by pushing up the tube Tu_1 by means of the handle H, which, when it is raised to the full extent is engaged by a turning movement in the rectangular catch C. Thus with any given adjustment the plate is locked in a position with the gap always the same between the bottom of the tower and the spray plate. With a gap of $\frac{1}{2}$ in. with the table up, there is a gap of $2\frac{1}{2}$ in. with it down, i.e. there is a 2 in. movement. Any necessary levelling of the spray table can also be done with the screws S_1–S_6.

The stand.

The whole apparatus is mounted in a rectangular wooden stand (Pl. 1 *c*), 41 in. high with sides $15\frac{1}{2}$ in. long. It consists of four wooden uprights of 2 in. cross-section. At both top and bottom there are four cross-pieces of the same material; on the top of the stand is a platform of five-ply with a hole in the centre through which the tower fits; the adjusting nuts through the top flange of the tower rest on brass pieces on this platform. The struts to take the base plate of the spray table mounting are fitted to the four uprights.

The compressed air supply.

The compressed air is supplied by an air compressor and is led to the nozzle through a system of filters and pressure regulators and stabilizers as indicated in the diagram (Text-fig. 3). Compressed air from the air receiver (AR) of the air compressor is led through the following units: (1) a separator and filter (S) where the main impurities of oil and water are removed, (2) a first valve (RV_1) to reduce the pressure to near that required, (3) a second valve (RV_2) further to reduce fluctuations in pressure, (4) to a small air receiver (AR_2) to equalize the flow, (5) through a tap (T), (6) through a needle valve regulator (NV) which finally stabilizes the pressure, (7) through a mercury pressure gauge (MG), and finally (8) through a sintered glass filter (SF) to remove the last trace of dust and dirt before reaching the nozzle. Pressure gauges G_1–G_3 are inserted in order to check the pressure. The elaborate arrangements for stabilizing the pressure are necessary when low pressures of the order of 20 cm. of mercury and less are used; also the slightest particle of dust or other matter is liable to stick in one side of the nozzle and interfere with the distribution and replication of the spray deposit.

Setting up the apparatus for use.

The tower is first levelled by means of the three adjusting screws in the top flange so that the plummet of a plumb-line suspended from the centre of the top falls in the centre of the bottom of the tower. The nozzle is then centred in the top of the tower and roughly levelled. The spray plate is centred over the bottom of the tower and levelled, so that the gap between it and the bottom flange is equal all the way round, this gap being then adjusted to the required distance. For final exact adjustment of the nozzle, it is necessary to do distribution tests with cover-slips, but the rough adjustment normally gives a fairly good distribution.

Physical performance of the apparatus

Method of testing.

When methods of testing the physical performance of the apparatus were first considered an attempt was made to adapt photometric methods for this purpose. A considerable amount of experimental work was done in spraying coloured solutions on to glass plates, the density and evenness of the deposit being measured by passing a beam of light through on to a photo-electric cell and measuring the current produced on a galvanometer. Various difficulties, however, were encountered, and it became obvious that much work would be necessary before a satisfactory practical technique could be hoped for. The method was therefore abandoned and the more laborious but more direct method of weighing was adopted. The

following procedures were used for measuring the errors of replication and the errors of distribution of the deposit.

For replication of deposit. The apparatus was set up for use and the pressure adjusted. A glass Petri dish 8·6 cm. diam. with walls 1·5 cm. high and covered with a ground plate lid was weighed and the Petri dish then placed on the spray table. 5 c.c. of the liquid to be tested was placed in the reservoir of the atomizer and sprayed through, the dish wiped, the lid put on and the whole reweighed. The deposit on the dish was thus obtained.

For distribution of deposit. A glass plate of 9 cm. was covered by a circle of paper which was marked out with a circle of 2·3 cm. diam. at the centre and three other similar circles equally distributed round the circumference. They were numbered 1, 2, 3, 4. Four small brass washers, each approximately 1 cm. diam., were placed at the centre of each of the circles.

Four cover-slips each of 2·3 cm. diam. were placed in numbered weighing bottles and weighed, and put on top of the washers in the circles on the glass plate. The glass plate was then placed on the spray platform and 5 c.c. of liquid sprayed on to it; it was then removed to a table, the cover-slips carefully lifted off with curved forceps, placed in their appropriate weighing bottles and reweighed. The plate was always put on the spray table so that no. 3 cover-slip on the circumference was toward the front of the apparatus. The washers served the purpose of raising the cover-slips so that none of the deposit would be drawn off the surface by the paper and they also facilitated lifting with the forceps. After each spraying the bottles and cover-slips were dried and cleaned carefully and the variation in the series shown in Table 2 between one set of weighings and the next may be in part due to lack of attention to this detail. The technique is laborious and subject to error, especially after some hours work.

Results of tests for errors of replication and distribution of deposit.

Distilled water was selected as the liquid since water besides forming the basis of all aqueous sprays is probably the most difficult liquid to atomize efficiently and distribute evenly.

Tables 1 and 2 give the figures for replication and distribution obtained in the apparatus finally decided on before it was set up for practical use. These figures were obtained working at very low pressures at which the replication might be at its best and the distribution at its worst.

TABLE 1. *Preliminary tests for replication of deposit*

Liquid used = distilled water. Amount used = 5 c.c.
Pressure = 12·92 cm. Hg. Room temperature = 64° F.
Room rel. humidity = 70 %. Gap between spray table and bottom of tower = 0·75 in.
Glass dish = 8·6 cm. diam. with walls 1·5 cm. high.

Weighing no.	Total deposit mg.	Deposit mg./sq. cm.
1	467·0	8·036
2	462·0	7·950
3	459·5	7·907
4	468·0	8·054
5	456·0	7·847
6	458·5	7·890
7	438·5	7·546
8	462·0	7·950
9	448·0	7·710
10	462·5	7·959

Mean total deposit = 458·2 mg. Area of dish = 58·11 sq. cm.
S.E. ± 8·944. Mean deposit per sq. cm. = 7·885 mg.
Proportional S.E. = 1·952 %.

TABLE 2. *Distribution preliminary trials; conditions as in Table* 1

Test no.	Position 1 (centre) mg.	Position 2 mg.	Position 3 mg.	Position 4 mg.	Means mg.
1	30·2	32·1	28·7	28·7	29·92
2	26·7	28·5	27·7	26·9	27·45
3	29·5	29·5	27·7	28·8	28·88
4	30·8	32·5	28·7	27·4	29·85
5	32·1	31·0	29·7	29·2	30·50
Means	29·86	30·72	28·5	28·2	29·32

S.E. of a single deposit $\pm 0{\cdot}98 = 3{\cdot}34\,\%$ of the mean.
S.E. of mean of five deposits at one position $= \pm 0{\cdot}44$.
S.E. of mean of four deposits at one trial $= \pm 0{\cdot}49$.

From the figures the replication of deposit appeared satisfactory. The distribution was fair, but the deposit on the cover-slips varied rather widely from one set of weighings to the next, partly because the determinations were carried out over two days, during which the atmospheric conditions varied, and partly to the difficulties of technique. The gap between the spray table and the bottom of the tower was $\frac{3}{4}$ in. for these determinations. The tower was now set up ready for practical use and a series of weighings were made to find out the relationship between the air pressure and deposit and how the replication of deposit varied at different air pressures. (Tables 3–8.)

TABLES 3–8. *Tests to show the variation in replication of deposit at different air pressures and the relationship between air pressure and deposit*

Liquid used = distilled water. Amount used = 5 c.c.
Room temperature = 74–76° F. Room rel. humidity = 45–52 %.
Glass dish 8·6 cm. diam. with walls 1·5 cm. high.
Gap between spray table and bottom of tower = 0·75 in.

TABLE 3. *Pressure* 11·31 *cm. Hg*

Weighing no.	Total deposit mg.	Time to spray through sec.	Deposit mg./sq. cm.
1	399·0	22	6·866
2	398·5	22	6·858
3	413·0	21·5	7·107
4	407·5	22	7·013
5	415·0	21·5	7·142

Mean total deposit = 0·4066 g. S.E. $\pm 0{\cdot}007677$.
Mean deposit per sq. cm. = 0·00699 g. Proportional standard error $= \pm 1{\cdot}888\,\%$.

TABLE 4. *Pressure* 19·47 *cm. Hg*

Weighing no.	Total deposit mg.	Time to spray through sec.	Deposit mg./sq. cm.
1	333·5	17	5·739
2	329·0	17	5·662
3	337·5	16·5	5·808
4	335·0	17·5	5·765
5	333·5	17	5·739

Mean total deposit = 0·3337 g. S.E. $\pm 0{\cdot}003094$.
Mean deposit per sq. cm. = 0·005742 g. Proportional standard error $= \pm 0{\cdot}9272\,\%$.

TABLE 5. *Pressure 27·20 cm. Hg*

Weighing no.	Total deposit mg.	Time to spray through sec.	Deposit mg./sq. cm.
1	292·5	14	5·034
2	310·5	14	5·343
3	304·5	13·5	5·240
4	307·0	13·5	5·283
5	301·5	14	5·188

Mean total deposit = 0·3032 g. S.E. ± 0·006834 g.
Mean deposit per sq. cm. = 0·005218 g. Proportional standard error = ± 2·254 %.

TABLE 6. *Pressure 37·78 cm. Hg*

Weighing no.	Total deposit mg.	Time to spray through sec.	Deposit mg./sq. cm.
1	276·5	11·5	4·758
2	290·5	11·5	4·999
3	281·5	11	4·844
4	289·7	11	4·985
5	271·0	11·5	4·664

Mean total deposit = 0·2818 g. S.E. ± 0·008410 g.
Mean deposit per sq. cm. = 0·004850 g. Proportional standard error = ± 2·984 %.

TABLE 7. *Pressure 60·20 cm. Hg*

Weighing no.	Total deposit mg.	Time to spray through sec.	Deposit mg./sq. cm.
1	213·5	9·5	3·674
2	215·5	9·5	3·708
3	227·0	9	3·906
4	226·0	8·5	3·889
5	224·0	9	3·855

Mean total deposit = 0·2212 g. S.E. ± 0·005863 g.
Mean deposit per sq. cm. = 0·003806 g. Proportional standard error = ± 2·651 %.

TABLE 8. *Relationship between air pressure and deposit*

Air pressure cm. Hg	Total deposit average mg.	Deposit mg./sq. cm.
11·31	406·6	6·997
19·47	333·7	5·743
27·20	303·2	5·218
37·78	281·84	4·850
60·20	221·2	3·807

Tables 3–8 show that a wide range of deposits can be obtained by variation of the air pressure and the replication of deposit was approximately the same for all the pressures tested. The pressure of approximately 20 cm. gave exceptionally small (replication) errors, but subsequent determinations showed that this was a chance result and that the normal proportional standard error is from 2 to 3 % throughout. The figures also show that the deposit is especially sensitive to changes in air pressure at low air pressures; hence the necessity, when working at low air pressures, of careful stabilization of pressure.

An air pressure of approximately 20 cm. was then decided on for a normal working pressure because it gave a sufficiently heavy deposit with the 5 c.c. of liquid normally used, and slight differences of air pressure were not so likely to be important as when a low pressure was used. A distribution test was done at this pressure and the figures are given in Table 9. In order to obtain these figures the gap was adjusted to $\frac{1}{2}$ in. and a further test was made to ascertain whether this had interfered with the replication, the results being given in Table 10. The figures for distribution when examined statistically show that under these conditions there is no significant variation of distribution over the area tested, which was a circle of 9 cm., i.e. the area used in practice.

TABLE 9. *Distribution* (*apparatus set up for use*)

Room temperature = 65° F. Room rel. humidity = 68 %.
5 c.c. distilled water. Pressure = 18 cm. Hg.
Gap between spray table and bottom of tower = 0·5 in.

Mg. per coverslip 2·3 cm. diam.

Time to spray	Position 1 centre	Position 2	Position 3	Position 4	Mean
sec.	mg.	mg.	mg.	mg.	mg.
17	26·0	24·4	24·8	26·2	25·35
16·5	23·2	24·5	24·9	24·0	24·15
16·5	23·9	24·7	25·0	24·1	24·42
16·5	23·6	25·0	25·1	25·2	24·72
16	24·0	25·8	26·0	24·2	25·00
Means	24·14	24·88	25·16	24·74	24·73

S.E. of a single deposit = 0·76 = 3·07 % of the mean.
S.E. of the mean of five deposits at one position = 0·34.
S.E. of the mean of four deposits at one trial = 0·38.

TABLE 10. *Replication under similar conditions*

Time to spray	Mg./dish 8·6 cm. diam.	Mg./sq. cm.
sec.		
16·5	354·5	6·100
16·5	370·5	6·376
16	351·0	6·040
16	348·5	5·997
16·5	355·5	6·118

Mean total deposit = 0·3560 g. S.E. ± 0·008573.
Mean deposit per sq. cm. = 0·006126 g. Proportional standard error = ± 2·408.

It appears therefore that under optimum conditions the apparatus is capable of repeating a given deposit with a proportional standard error of 2–3 % and giving at the same time a distribution where no significant difference in evenness is shown by the method of demonstration used.

Since much work has been and is being done on insecticides carried in a petroleum oil medium, a few tests were done on the replication of deposit, using first a light oil of the fly spray type and secondly a heavy white oil of the type used as an industrial insecticide in warehouses. No distribution tests were done with these oils, since, as water is far more difficult to distribute evenly, the distribution of the oils might be expected to be as good or better than that obtained with distilled water.

TABLE 11. *Replication using a light oil*

Room temperature = 64° F. Room rel. humidity = 70 %.
Pressure = 17·97 cm. Hg. Amount in reservoir = 5 c.c.
Glass dish = 8·6 cm. diam. with walls 1·5 cm. high.
Gap between spray table and bottom of tower = 0·5 in.

Weighing no.	Time to spray sec.	Total deposit mg.	Deposit mg./sq. cm.
1	20·5	251·0	4·319
2	21·0	263·0	4·526
3	20·5	259·5	4·466
4	21·0	252·5	4·345
5	21·0	253·0	4·354
6	21·0	252·5	4·345
7	20·75	255·5	4·397
8	21·0	245·5	4·225
9	21·5	249·5	4·294
10	21·5	252·5	4·345

Mean total deposit = 0·25345 g. S.E. ± 0·004906.
Mean total deposit per sq. cm. = 0·004362 g. Proportional S.E. = ± 1·936.

The light oil used has the following specification: sp.gr. 0·779; flashpoint closed, 158° F.; visc. redw. 1 at 60° F., 32 sec.; initial boiling-point, 200° C.; final boiling-point, 270° C. Table 11 shows the results of replication tests under similar conditions to those under which water sprays were tested and the errors are very similar.

TABLE 12. *Replication using a white oil*

Room temperature = 71° F. Room rel. humidity = 52 %.
Pressure = 33·64 cm. Hg. Amount in reservoir = 5 c.c.
Glass dish = 8·6 cm. diam. with walls 1·5 cm. high.
Gap between spray table and bottom of tower = 0·5 in.

Weighing no.	Time to spray sec.	Total deposit mg.	Deposit mg./sq. cm.
1	77·5	115·5	1·988
2	77·5	115·0	1·979
3	78·0	118·5	2·039
4	77·5	118·5	2·039
5	77·5	119·0	2·048

Mean total deposit = 0·1173 g. S.E. ± 0·001890.
Mean total deposit per sq. cm. = 0·002018 g. Proportional S.E. = ± 1·611 %.

The heavy white oil used has the following specification: sp.gr. 0·862; flashpoint closed, 320° F.; flashpoint open, 335° F.; visc. redw. 1 at 70° F., 118 sec.; pour test = − 30° F. Unsulphonatable residue, 99·2 %. This oil which is nearly odourless and tasteless is used in industrial insecticides in warehouses for the protection of stored foodstuffs; it is much more viscous than the light oil and atomizes very slowly at the air pressure used for water sprays, so the pressure was raised and a short series of tests done. Results are given in Table 12. In this short series the errors were smaller than with either water or a light oil, but it is probable that with a longer series the errors would increase and be of the same order as that of the series obtained with the other two. The nozzle appeared to atomize both the oils efficiently.

Adjustment of the amount of deposit.

The figures show the possibility of obtaining any deposit that is likely to be required. Two methods are available for adjusting the amount of material deposited on the spray plate; viz. by variation of the amount of liquid in the reservoir and by variation of the air pressure. Major alterations are best effected by the first method and the final adjustment made by the second. By using the needle valve air-stabilizer, the air pressure can easily be adjusted with considerable accuracy and the deposit regulated to within a few milligrams of any desired amount.

There is no doubt that the total weight of deposit is subject to variation from day to day or even between morning and afternoon, even while the air pressure and all the other adjustments are kept as standard as possible. This variation may be due to changes in atmospheric conditions or to slight changes in the adjustments, the most delicate and sensitive of which is the nozzle itself. The deposit should therefore always be checked before the apparatus is used and rechecked if there is any marked change in atmospheric conditions such as temperature and humidity. The checking only takes a few minutes and enables the deposit to be adjusted with a considerable degree of accuracy.

Test insects and rearing methods

If it is desired to control a particular pest by means of insecticides, the only satisfactory method is to make laboratory and field trials on the species causing the damage, but in order to make a general assessment of the value of any substance as an insecticide, it may be sufficient in the first instance to test it on one or two species of insects. The chief desirable characters apart from any questions of susceptibility of a test subject are: (1) That it can be reared continuously throughout the year without any seasonal rhythm. (2) That the life cycle should be short. (3) That as many instars as possible should be suitable for test subjects. (4) That the stage tested should be able to do without food during the period of spraying and observation. Where plant feeding insects are concerned, to these must be added that the plant may be easily reared throughout the year in a condition to support the insect at the required instar.

Although specificity of effect renders difficult the task of recognizing and comparing an insecticide with others, it may not be so hopeless as it might appear. Some species of insects appear, in general, to be highly susceptible and others highly resistant, and although the order of susceptibility may change with different insecticides, the changes do not appear to be so great as to prevent a general classification of insects into groups according to their susceptibility. Some work on this point has been carried out in this laboratory and will be published later. So far as can be ascertained at present, the species of aphides, used for test purposes, have proved susceptible to all substances of any insecticidal value, so that these insects may be regarded, with comparative safety, as being generally susceptible. It is therefore probable that any substance that will not kill these aphides will not be a useful insecticide for immature and adult insects though it is possible that ovicides may not be detected by these methods. For the recognition and preliminary study of an insecticide substance, a suitable species of aphid should be readily and continuously available.

The species chosen for this purpose was the black chrysanthemum aphis, *Macrosiphoniella sanborni* Gill. It cannot be definitely stated that this is the best species for the purpose, but all the information available suggested it as the most likely to give results. It has been found

that this insect may be reared throughout the year, that its resistance is sufficiently high without being too high, and that it is very easily handled. So far, not enough work has been done on the rearing of this species to enable detailed instructions to be given, but the three main points to be observed are: (1) to maintain a stock of healthy chrysanthemum plants throughout the year, (2) to select one or more suitable varieties of plants. Some varieties are much better than others for the purpose of rearing the insects, but so far it is not possible to list the best varieties. In general, they appear to be those with the most sappy growth, the greenhouse varieties being much better than the outdoor varieties. (3) Periodically, healthy individual insects should be selected and isolated from the stock, which is then destroyed and fresh cultures made up from the selected individuals. The times at which this is best done are not fixed, but can easily be judged by the appearance in the stocks of large numbers of undersized, unhealthy looking individuals, for if the stock is then allowed to go on without interference, it degenerates until all the individuals are undersized and usually a very high percentage of undersized winged forms are produced. If, however, the selection procedure is adopted, it appears possible to maintain the stocks in a good condition over a greater part of the year, if not the whole year, and where the stocks have appeared to be in a good condition, very little variation in level of resistance of the population has been found. On some occasions the insects used for one experiment have been raised from a single individual, but no greater homogeneity has been observed than when the stock was started from several individuals, though the inbred stock has not been compared with wild colonies. The adult wingless parthenogenetic viviparous insects are used as test subjects.

During the summer months *Aphis rumicis* L., the black-bean aphis, is a very good species for testing purposes and is a useful addition to *Macrosiphoniella sanborni*, but so far it has only been reared satisfactorily over a short period in the summer, and this period is very uncertain both in duration and in time of the year. Attempts to prolong the period during which adult wingless parthenogenetic viviparous females, the stage used for testing, are present, have proved unsuccessful. The method at present adopted is to grow bean plants in pots which are covered with muslin. When the aphides appear on beans in the open, individuals are taken and placed on the beans in pots and are kept breeding until the normal migration sets in. Judging on the available data the resistance level of *Aphis rumicis* is higher than that of *Macrosiphoniella sanborni*.

Once a substance has been shown to have insecticidal value, it is frequently desirable to test it on other more resistant species of insects, often for the purpose of comparison with other insecticides and to test it on the various instars. For this and other types of insecticide work, it is desirable to have a number of other species of insects available.

Two other species of plant-feeding insects are being reared in this laboratory which show considerable promise as test subjects of greater resistance than the aphides; these are *Plutella maculipennis* Curtis, the diamond back moth, and *Dysdercus intermedius* Dest., a species of cotton stainer. Both these species have been reared continuously without any serious difficulty over two years, and it appears possible under suitable conditions to rear a generation in about six weeks. Very little insecticidal work has yet been done with these two insects. The adult cotton stainer and the fully grown larva of *Plutella maculipennis* both appear to be very resistant to derris insecticides although these are frequently recommended against the latter species. Only preliminary trials have been made on the other instars, but the eggs of *P. maculipennis* appear to be promising test subjects. Until considerably more

insecticidal work has been done to ascertain the full value of these insects as test subjects and more work has been done on their biology, it does not appear worth while to give details of rearing technique.

Owing to the ease with which some of the insects which attack stored goods can be reared in large numbers, attention has so far been concentrated on them as the source of supply of species of higher resistance level than aphides. Such insects can have the following advantages: (1) a large number of generations is produced in the year without any seasonal rhythm; (2) the difficulty of rearing plants is dispensed with, thus making a greater degree of standardization of the conditions possible; (3) a large number of insects can be produced in a small space with the minimum of labour, so that even with space and labour severely restricted, several species with different resistance levels can be reared. Preliminary tests were carried out on twenty species of stored product insects, and it was decided that the most suitable for general work was *Oryzaephilus surinamensis* L., the saw-toothed grain beetle. Several others are thought to be very useful and sixteen species are kept going, some of which have been used from time to time, though they have not yet been thoroughly investigated.

O. surinamensis, the saw-toothed grain beetle, is reared in a room kept at 27° C. and 70% relative humidity, on a diet of rolled oats and sultanas. The food is first fumigated with carbon disulphide to get rid of predacious mites and is then aired in a fume cupboard for at least 24 hr. Glass jars 8 in. high and 4 in. diam. are used for each culture, four cultures usually being started at one time. Food is placed in the jar to a depth of about 4 in. and then 100 adults are placed in the jar which is covered by a muslin top and put in the constant temperature room. Under these conditions the length of the life cycle from the placing of the adults in the jar to fresh adults emerging is approximately one month. When kept on food at 27° C. and 70% relative humidity, the adult beetle normally lives at least six months. Twenty-one out of twenty-five insects, which were isolated on emerging and placed on sultanas in tubes, were still alive at the end of that period. The adults are, however, very susceptible to starvation and die in 3–6 days when kept without food.

The adults of *Oryzaephilus surinamensis* have proved very useful test insects; they are susceptible to moderate concentrations of derris products, pyrethrum, nicotine, and lauryl thiocyanate, and have proved resistant to any of the carrying media so far tried except where a heavy deposit of oil was used; they were resistant to a light deposit. Other adult insects which seem to be of the same order of resistance as *O. surinamensis* L., are *O. mercator* Fauv., *Ahasverus advena* Waltl., *Lasioderma serricorne* F. and *Laemophloeus turcicus* Grouv. Crauford-Benson (1938 *b*) preferred *Ahasverus advena* as the most suitable test insect from a range of species which was similar to that tried here. He included *Oryzaephilus surinamensis* and *O. mercator*, as being on an equal footing with *Ahasverus advena*, but did most of his work with *Ahasverus*.

There does not appear to be any great difference in the resistance level between the three species, but *A. advena* feeds on moulds, and it was thought that it would be very much more difficult to produce standard conditions in mouldy cultures than in clean ones and for this reason the *Oryzaephilus* spp. were preferred. So far as could be ascertained, there is no reason for preferring *O. surinamensis* to *O. mercator*, but the former was available in considerable numbers at the start of the tests and its use was therefore continued. The relative resistance levels of the three species of insect most used by us, i.e. *Macrosiphoniella sanborni*,

Aphis rumicis and *Oryzaephilus surinamensis* is shown in Tables 19–21 and Text-fig. 5, graphs 6–8. These figures were obtained using pure rotenone as an insecticide.

It is recognized that it is desirable to have available a test insect from all the main natural orders and to be able to do tests on every instar in all these orders; furthermore, that there should be representatives of plant-feeding insects, insects affecting public or animal health and those of industrial importance. This could be done, but would involve much labour and expenditure, which would scarcely be justified at the present stage of our knowledge of the biological action of insecticides. Insects being bred at the moment include representatives of the Rhynchota, Lepidoptera and Coleoptera, and it appears probable that a number of species in each of these three orders will be found which can be reared easily and which can provide test subjects in every instar.

Factors causing variation in dosage-mortality data

It seems to be generally admitted that it is difficult, if not impossible, to make a complete statement of a set of conditions in which a given concentration of a drug at a given dosage will kill a given percentage of a given population of organisms, yet some attempt at this statement has to be made before a reliable technique for obtaining dosage-mortality data can be elaborated. The sources of variation in the determination must therefore be found out as far as possible and their relative importance assessed. A number of workers have published data on one or more of these sources of variation and it is possible to form a general estimate of some of the factors that appear most important, but there is nowhere the assumption that all the factors causing variation have been recognized. It is generally conceded that the best that can be done at the moment is to standardize the conditions of testing as rigidly as possible in the light of existing knowledge and to rely on statistical methods to decide on the significance of the results. In the absence of any complete statement of the sources of error they may be classified under the three heads: (1) errors due to unequal administration of the dose, (2) errors due to variation in insect resistance, (3) errors due to variations in the general technique such as handling and treatment before and after administration of the dose.

Errors due to unequal administration of the dose.

Before any estimates of variations due to other causes can be made, it is necessary to find a technique whereby an equal dose can be administered to each test insect, but owing to the magnitude of the other errors, it is difficult to assess the effect of variation of dosage alone on insect mortality.

All the testing methods available at present both for sprays and fumigants give dosage-mortality data which show a considerable variation. Perhaps the most accurate method of applying a known dosage of liquid contact insecticide is the micropipette method first used by O'Kane *et al.* (1933) and elaborated by Nelson *et al.* (1934). The biological data given by these latter authors, although very constant when averaged, showed considerable variation within each set of tests with a given insecticide at a given dose. Some data on this point were obtained with the apparatus described in this paper. The results of the first series of experiments are given in Table 13. In these experiments the dishes containing the insects were weighed before and after spraying, so that the weight of the deposit was known accurately. The table indicates that under the conditions of the experiment where the concentration of the poison is below a certain threshold value, variation of the deposit within wide limits has

little or no effect. (The experiment was done in a bare glass dish and the heaviest deposit was only a little short of that at which death due to physical causes was likely to occur.) Where, however, there is a concentration of poison above this threshold value, the percentage mortality increases with increase of deposit. It will be seen, however, that the results are very variable and they indicate that there are factors to be considered which either have not been recognized or the relative importance of which is not appreciated.

TABLE 13. *Effect of weight of deposit on mortality*

Test insect: *Oryzaephilus surinamensis* adults.

Insecticide = W. 212 *Derris malaccensis* resin suspended in water with 5 % ethyl alcohol and 0·25 % sulphonated lorol.

Insects sprayed on a bare Petri dish 9 cm. diam. which was weighed before and after spraying.

(*a*) 0·001 % resin			(*b*) 0·002 % resin		
Deposit on dish mg.	% badly affected and dead 1st day	% badly affected and dead 2nd day	Deposit on dish mg.	% badly affected and dead 1st day	% badly affected and dead 2nd day
80·5	3·5	8·9	45·5	38·8	26·0
81·0	13·2	2·6	49·5	40·0	26·7
87·5	16·7	9·6	51·0	19·0	23·8
101·0	9·6	6·0	55·5	58·8	39·6
109·0	24·4	6·8	60·0	69·8	47·2
113·0	9·2	12·2	62·0	64·3	39·0
123·5	17·6	2·0	63·0	44·9	14·3
138·5	14·0	8·2	69·0	35·6	18·2
			73·0	94·0	72·0
			73·5	75·0	60·8
			81·5	95·7	66·0
			94·5	40·0	15·1
			102·0	92·5	69·2

Owing to this condition of variation in mortality at a given deposit over successive sprayings, in order to make even an approximate assessment of the variation of mortality with deposit over a range of deposits, a number of experiments have to be made at each point and a curve plotted. The standard technique for obtaining dosage mortality curves in this laboratory is to have five replicas for each point. Curves have been obtained by Callaway & Musgrave (1940) using the apparatus first mentioned in this paper and shown in Pl. 8 *a*. These authors found that with their technique, if they converted the weight of deposit into logs and converted the mortality into probits, they obtained a straight line relationship between increase of deposit and increase in mortality analagous to that between increase in concentration and increase in mortality.

Some further data on the relationship between deposit and mortality are given in Tables 14–18 and graphs 1–5, Text-fig. 4. In these experiments two different techniques were used; in the first, the insects (adult *Oryzaephilus surinamensis*) were placed in bare glass Petri dishes and sprayed, while in the second, circles of tricholene were put in the Petri dishes to cover the bottom and the insects put on the cloth and sprayed. With the deposit kept constant, the toxicity at different concentrations of poison was determined and a curve drawn, the deposit was then altered and similar data obtained, this procedure was adopted for a number of different deposits using the two techniques outlined above.

Where the bare dishes were used, it was found that the curve for a deposit of 85 mg. showed a reduction in mortality at any given concentration from that given with a deposit

TABLES 14–18. *Effect of variation in deposit on percentage mortality and effect of variation in technique on mortality*

Test insects: adult *Oryzaephilus surinamensis* L.

(a) Insects placed in bare glass dish 9 cm. diam.

TABLE 14

Deposit approximately 80 mg. (Av. deposit 78·1 mg. = 1·23 mg./sq. cm.)
Carrying medium = 0·5 % saponin solution in water + 10 % ethyl alcohol.
Insecticide = W. 214 Derris resin (*Derris elliptica* Changi).
Temp. = 61° F. Rel. humidity = 58·6.
Date of trial 22 Feb. 1940.

Conc. of resin mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	58	0·0	0·0	
10	1·0000	60	0·0	0·0	
20	1·3010	63	4·8	3·3354	$n = 4$
40	1·6021	63	1·6	2·8556	$\chi^2 = 1·35$
80	1·9031	63	25·4	4·3380	for $P = 0·05$
120	2·0792	52	53·9	5·0979	$\chi^2 = 9·49$
160	2·2041	60	78·3	5·7824	
240	2·3802	56	96·4	6·7995	

TABLE 15

Deposit approximately 80 mg. (Av. deposit 85·0 mg. = 1·34 mg./sq. cm.)
Carrying medium = 0·5 % saponin solution in water + 10 % ethyl alcohol.
Insecticide = W. 214 Derris resin (*Derris elliptica* Changi).
Temp. = 60° F. Rel. humidity = 58·0 %.
Date of trial 13 Mar. 1940.

Conc. of resin mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	61	4·9	—	
40	1·6021	65	14·5	3·9419	
60	1·7782	58	43·8	4·8440	$n = 5$
80	1·9031	60	59·7	5·2456	$\chi^2 = 11·33$
100	2·0000	67	92·2	6·4187	for $P = 0·05$
120	2·0792	69	86·3	6·0939	$\chi^2 = 11·070$
160	2·2041	57	96·3	6·7866	
240	2·3802	88	100·0	[7·7501]	

TABLE 16

Deposit approximately 150 mg. (Av. deposit 155 mg. = 2·44 mg./sq. cm.)
Carrying medium = 0·5 % saponin solution in water + 10 % ethyl alcohol.
Insecticide = W. 214 Derris resin (*Derris elliptica* Changi).
Temp. = 60·7° F. Rel. humidity = 58·0 %.
Date of trial 13 Mar. 1940.

Conc. of resin mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	70	2·86	—	
20	1·3010	66	23·5	4·2808	
40	1·6021	62	56·8	5·1713	$n = 5$
50	1·6990	60	69·1	5·4987	$\chi^2 = 15·93$
60	1·7782	72	94·3	6·5805	for $P = 0·05$
70	1·8451	66	96·9	6·8663	$\chi^2 = 11·070$
80	1·9031	60	100·0	[7·2551]	
100	2·0000	56	96·3	6·7866	

(*b*) *Insects placed in a glass dish 9 cm. diam. containing a circle of tricholene*

TABLE 17

Deposit approximately 150 mg. (Av. deposit 150·0 mg. = 2·36 mg./sq. cm.)
Carrying medium 0·5 % saponin solution in water + 10 % ethyl alcohol.
Insecticide = W. 214 Derris resin (*Derris elliptica* Changi).
Temp. = 64° F. Rel. humidity = 60 %.
Date of trial 3 Apr. 1940.

Conc. of resin mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	113	1·77	—	
90	1·9542	104	45·2	4·8794	
100	2·0000	129	51·1	5·0276	$n = 5$
110	2·0414	120	77·9	5·7688	$\chi^2 = 9·57$
120	2·0792	129	73·2	5·6189	for $P = 0·05$
130	2·1139	107	87·6	6·1552	$\chi^2 = 11·070$
140	2·1461	112	88·2	6·1850	
160	2·2041	98	94·8	6·6258	

TABLE 18

Deposit approximately 300 mg. (Av. deposit 309·5 mg. = 4·86 mg./sq. cm.)
Carrying medium 0·5 % saponin solution in water + 10 % ethyl alcohol.
Insecticide = W. 214 Derris resin (*Derris elliptica* Changi).
Temp. = 64° F. Rel. humidity = 60 %.
Date of trial 3 Apr. 1940.

Conc. of resin mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	102	0·98	—	
50	1·6990	116	26·83	4·3811	
60	1·7782	103	50·00	5·0000	$n = 5$
65	1·8129	107	50·91	5·0226	$\chi^2 = 9·76$
70	1·8451	118	70·03	5·5244	for $P = 0·05$
80	1·9031	108	90·65	6·3225	$\chi^2 = 11·070$
90	1·9542	100	96·96	6·8808	
110	2·0414	116	100·0	[7·5490]	

of 150 mg. These two curves were obtained using insects from the same culture, sprayed on the same day and with the conditions otherwise the same. A curve obtained for a deposit of 78 mg. showed a reduction in mortality from that in which 85 mg. were used but this curve was obtained with a different culture of insects on a different day under different conditions. This reduction appears too great to be accounted for by the decrease in deposit which is very slight and serves to indicate the variation likely to occur from one week to the next and to emphasize the necessity for care before comparisons are made. With the dishes containing tricholene similar results were obtained, the series with a deposit of 300 mg. showing a marked increase in toxicity over that with a deposit of 150 mg., the conditions otherwise being the same.

These experiments also indicate that if the technique is altered the effect of a given deposit is altered. A deposit of 150 mg. in a bare dish gave a much greater mortality at any given concentration than 150 mg. in a dish containing tricholene. The experiments are not strictly comparable, but taken together with others this point is established with a high degree of certainty. In the instance mentioned the reduction in kill when the dishes contain tricholene can be explained by the absorption of surplus spray by the cloth.

From this evidence it is clear that there can be no satisfactory basis for exploring the effect of liquid insecticides until a technique has been worked out for ensuring that each insect receives a known dose of poison. For this reason most of the work described in this paper has been devoted to devising an apparatus capable of delivering an equal dose of insecticide to each test insect and at the same time to treating, without undue fatigue, a number of insects sufficiently large for statistical analyses.

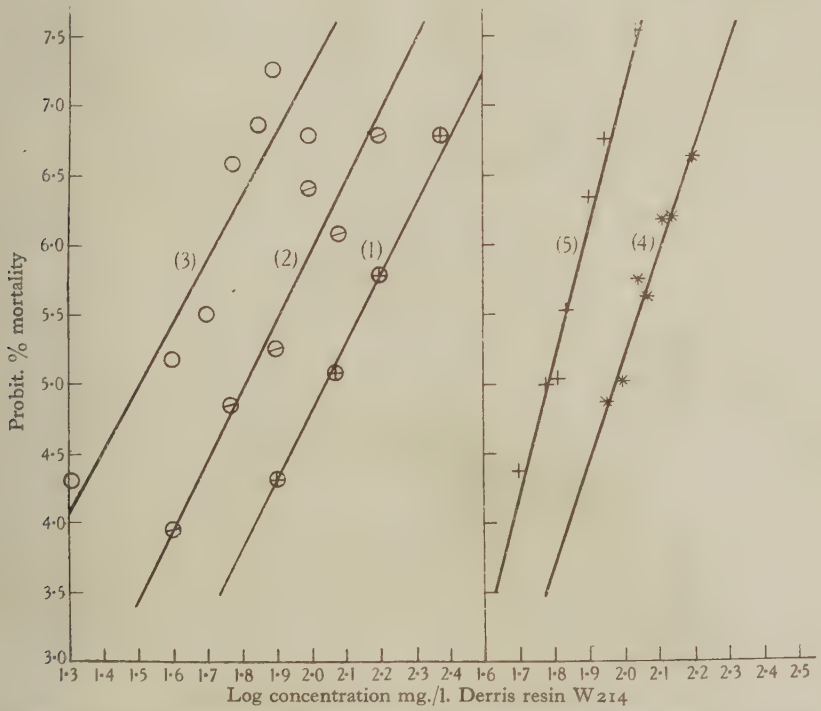


Fig. 4. Graphs nos. 1–5 showing effect of deposit on mortality and effect of technique on mortality. Test insect adult *Oryzaephilus surinamensis*, insecticide Derris resin W214. (1) 78 mg. deposit on bare dish; (2) 85 mg. deposit on bare dish; (3) 155 mg. deposit on bare dish; (4) 150 mg. deposit on dish with tricholene; (5) 309·5 mg. deposit on dish with tricholene. (See Tables 14–18 and text, pp. 160–3, 165.)

Errors due to variation in insect resistance.

Variation in insect resistance may be from one individual to another or from one population to another. Several workers have investigated the sources of individual variation and it has been established with a greater or less degree of certainty that with a given instar of a given species the resistance may vary according to the age and sex of the individuals. The wide individual variation that is found to occur when these two factors are standardized can only be accounted for by what may be termed heredity factors, and there is so far no information adequate to show whether or not this individual variation can be reduced by suitable selection. Nelson *et al.* (1934) found a slight improvement in uniformity through inbreeding from a single pair without any loss of resistance. In this laboratory the aphides used for test purposes have frequently been raised from a single individual, but no com-

parative data have been obtained to show that this results in any greater uniformity in individual resistance than when this technique was not adopted. So far no work has been done to obtain homozygous test insects and to investigate the effect of this on variation of resistance.

It appears certain that the resistance of the population as a whole may change its level to a given poison in a few generations. The reasons for this change of level are not known. Presumably, if it were possible to rear the insects under identical conditions, a stable equilibrium would be reached, but since factors such as population density appear to influence the resistance this is not an easy matter, so that, although by a rigid standardization of rearing technique this variation may be reduced, it must always be recognized as liable to occur. It is possible to approximate to standard conditions of rearing only with insects attacking stored goods. With plant insects the conditions are influenced by the stage of growth of the plant and the conditions necessary for plant health and they are therefore much more difficult to standardize. In this laboratory the insects attacking stored goods are reared in a room kept at a constant temperature and humidity and with the other conditions such as food and population density kept reasonably constant. No attempt has been made to standardize the conditions of rearing of the plant insects; attention has been entirely directed to providing healthy plants in conditions which are to them unnatural and to discover which of these conditions favour the continuous production of healthy insects. Over the period during which our experiments have been made, although some alteration in resistance levels has been observed, there have been no major changes when insects were used which have been carefully reared and which appeared healthy, judged by external appearance.

Errors due to variation in general technique.

In addition to a slow change in the resistance of the population from one generation to the next, differences in resistance may be produced by more immediate conditions of pre-treatment. If insects of a given population are divided into batches and are kept at different temperatures for some time before treatment, there is some evidence to show that those kept at the higher temperatures are less resistant than those kept at the lower. The effect of humidity does not appear to be so noticeable (Gough, 1939). It also appears that the period of removal from their food before treatment has some effect and this should be standardized as far as possible (Gough, 1939; Crauford-Benson, 1938 *a*). No assessment has been made of the effect of handling the insects, but it is quite possible that this may have some effect on their resistance and it is very difficult to ensure that each insect is treated equally in this respect.

There is evidence to show that conditions of after-treatment are also important in affecting the resistance. Here again temperature is the factor chiefly insisted upon, but it would seem that when testing contact sprays in aqueous media, humidity may also play a large part since it affects the rate of evaporation of the carrying medium. Presumably a low humidity causing a rapid rate of evaporation may reduce the effect of the poison, due to its being thrown out of suspensions or solution in the carrying medium and thus preventing penetration into the organism. The humidity of the microclimate around the insects is greatly influenced by the nature of the container in which the test insects are kept after spraying and it is suggested that precautions should be taken to ensure that this microclimate is kept the same in every case.

The effect of variation in the general technique is illustrated by the data set out in Tables 14–18 and the curves shown in Text-fig. 4, graphs 1–5. In the earliest experiments with small insects such as *Oryzaephilus surinamensis* L., *Ahasverus advena* Watl. and *Laemophloeus turcicus* Grouv. a glass Petri dish was used in which the insects were sprayed; they were then kept in the dish until examined. Using a carrying medium of water containing from 5–10% of ethyl alcohol or acetone and either 0·5% saponin or 0·25% sulphonated lorol, it was found that with deposits of much more than 80 mg. per dish of 9 cm. diam., many of the insects tended to stick to the dish. Nearly all the insects so affected were dead and it is not possible to state whether this was partly responsible for their death or not. Some experiments were carried out to compare the effect of spraying *Oryzaephilus surinamensis* in a bare Petri dish with one in which a circle of absorbent material (tricholene) covering the floor of the dish had been placed. In the bare dishes deposits of approximately 80 mg. and approximately 150 mg. and in the dishes containing tricholene of approximately 150 and 300 mg. were tested. The data obtained show the very great effect of an alteration of some factors in the physical environment of the insect during and after spraying when all the other conditions such as dosage, concentration, temperature and humidity are kept the same. The addition of the circle of tricholene not only completely prevented sticking, but compared with a bare dish, it greatly decreased the mortality at a given dose and concentration and altered the slope of the dosage mortality curve, the slope being steeper when dishes containing tricholene were used. The reason for these changes are not clear; reduction in mortality at a given dose is probably due to absorption of the excess spray fluid by the cloth, but it is not easy to understand why the curves are steeper when tricholene is used.

The results using a bare dish with a deposit of 150 mg. gave a high χ^2 value and this heterogeneity was probably due to the extent to which the insects were stuck to the dish. By using a circle of tricholene, this difficulty was prevented, the test was made more sensitive and a very wide range of deposits might be used. This technique was therefore adopted.

The considerable difference in mortality that can be obtained with a given concentration of poison by varying the conditions of spraying such as the weight of deposit and nature of environment, emphasize the futility of translating directly the results of laboratory experiments to recommendations for field use and of attempting to compare the toxicity of insecticides that have not been tested under conditions which are as nearly identical as possible. With any technique using spraying or dipping methods it is difficult to ensure that each insect remains under comparable conditions after treatment. It may remain on a surface wet with the poisonous liquid or crawl on to a part of the container which is dry and it does not appear possible to arrange for complete uniformity of environment after spraying.

The factors outlined are recognized or suspected of causing variation in response to poisoning, but there may also be others of great importance which have not so far been discovered. The most that can be done at present is to standardize the technique as much as possible, and by this means to attempt to control the important variables, both known and unknown.

Spraying technique

The technique at present adopted varies slightly with the species of insect. It is not possible to state rigid rules which will apply to all species of insects since some alterations may be necessary with different species and for different instars, but two examples, those of

the insects most frequently used in this work, should make clear the procedure in all its essential points.

(*a*) *For aphides, e.g.* Macrosiphoniella sanborni *Gill. and* Aphis rumicis *L.*

(1) In all tests the adult parthenogenetic viviparous females are used. The insects are removed from the plant and placed in glass tubes a few hours before spraying, ten individuals to a tube. With *Aphis rumicis* the plants are cut the previous day and placed in a large glass jar overnight; by the morning a large number of the individuals required are wandering and are transferred to tubes by means of a camel hair brush. If the insects are removed directly from the plant to the tube while still feeding, there is liable to be risk of injury to the insect by damaging the mouth parts. With *Macrosiphoniella sanborni* it is only necessary to tap the plants for the individuals required for test to fall off. They are thus easily collected in a container from which they are transferred to tubes. By these methods adequate randomization is secured if there are large numbers of insects in good condition, but a further randomization is made by mixing the tubes.

(2) The nozzle of the apparatus is centred and the gap at the bottom checked. About 25 c.c. of the control solution is then sprayed through to get an equilibrium set up in the apparatus and the deposit is checked by weighing. Once the apparatus has been set up and standardized, it is usually only necessary to do from three to five weighings to obtain the required deposit and to check it. A standard Petri dish is used, 8·5 cm. diam. with a ground-glass cover. For most general purposes, 5 c.c. of insecticide are placed in the reservoir and the pressure adjusted to give deposits of the order of 300–350 mg. in the apparatus described, this requiring an air pressure of 14–16 cm. of mercury, using a gap of 0·5 in. between the bottom of the tower and the spray plate.

(3) The insects are transferred from the tubes to Petri dishes containing circles of tricholene covering the bottom. They are then placed in the apparatus and sprayed. The spraying is usually replicated five times for each concentration, three replicates being a minimum.

(4) After spraying the dishes are removed, a leaf of the food plant added and the top of the dish is covered with muslin which is held by a rubber band. The dishes containing the sprayed insects are put on trays and removed to a cool damp place, usually a basement room keeping a relatively constant temperature. The insects are inspected the following day and classified as normal, slightly affected, badly affected, moribund, and dead. Fresh food is added and a second inspection is made the day after, if necessary.

Tables 20–21 and Text-fig. 5, graphs 7, 8, show the type of biological data obtained by this technique.

(*b*) *For insects attacking stored products e.g.* Oryzaephilus surinamensis *L.*

(1) Cultures are selected which are between 30 and 40 days old; it is estimated that in such cultures any individual adult should not be more than a week old. The adults are removed into tubes, usually twenty insects to each tube on the day before the experiment.

(2) and (3). The procedure is the same as with aphides.

(4) After spraying, the dishes are covered with a muslin cover held in place by a rubber band (no food is added) and removed to the constant temperature and humidity room, each

TABLES 19–21. *Typical curves obtained with the final technique described and showing the resistance levels of* Oryzaephilus surinamensis *L.*, Macrosiphoniella sanborni *Gill. and* Aphis rumicis *L. to pure rotenone*

Insecticides used: pure crystallized rotenone.

TABLE 19

Test insect: adult *Oryzaephilus surinamensis* L.

Deposit approximately 300 mg. (Av. deposit 311 mg. = 4·89 mg./sq. cm.)
Carrying medium = 0·5 % saponin solution in water + 10 % ethyl alcohol.
Temp. = 61° F. Rel. humidity = 52 %. Date of trial 10 Apr. 1940.

Conc. of rotenone mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	87	3·5	—	
9·9*	0·996	92	3·1	3·1337	$n=3$
21·0	1·322	93	3·0	3·1192	$\chi^2=5·11$
30·9	1·490	94	11·4	3·7945	for $P=0·05$
40·8	1·611	98	32·3	4·5407	$\chi^2=7·815$
51·9	1·715	93	60·0	5·2533	
61·8	1·791	96	86·1	6·0848	

* Point omitted from calculation of χ^2 and the regression line.

TABLE 20

Test insect: *Aphis rumicis* L. (viviparous parthenogenetic females).

Insects placed in a glass dish 9 cm. diam. containing a circle of tricholene.
Deposit approximately 300 mg. (Av. deposit 327·4 mg. = 5·14 mg./sq. cm.)
Carrying medium = 0·5 % saponin solution in water + 10 % ethyl alcohol.
Date of trial 9 July 1940.

Conc. of rotenone mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	49	4·08	—	
6	0·7782	48	13·1	3·8783	
8	0·9031	52	29·8	4·4698	
9	0·9542	49	21·3	4·2039	
10	1·0000	50	37·4	4·6787	$n=8$
11	1·0414	52	45·9	4·8970	$\chi^2=5·11$
12	1·0792	54	42·1	4·8007	for $P=0·05$
14	1·1461	50	58·4	5·2121	$\chi^2=16·919$
16	1·2041	48	58·7	5·2198	
18	1·2553	50	70·8	5·5476	
20	1·3010	49	80·9	5·8742	

TABLE 21

Test insect: *Macrosiphoniella sanborni* Gill (viviparous parthenogenetic females).

Insects placed on a tricholene circle, then sprayed and the tricholene circle placed in a 9 cm. Petri dish and the dish covered with damp cellophane.
Deposit approximately 350 mg. (Av. deposit 348·0 mg. = 5·47 mg./sq. cm.)
Temp. = 65° F. Rel. humidity = 75 %. Date of trial 16 July 1940.

Conc. of rotenone mg./l.	Loc. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	49	2·04	—	
2	0·3010	47	2·2	2·9859*	
4	0·6021	51	32·0	4·5323	$n=5$
6	0·7782	51	58·0	5·2019	$\chi^2=4·7$
8	0·9031	50	63·3	5·3319	for $P=0·05$
10	1·0000	50	77·6	5·7588	$\chi^2=11·07$
12	1·0792	51	90·0	6·2816	
14	1·1461	49	93·8	6·5382	
16	1·2041	51	100·0	7·0754	

* Point not used in calculating χ^2 and the regression line.

set of five is removed to the room before spraying the next set. They are inspected on the following day and a second inspection is made if necessary the day after.

Tables 17–19 and Text-figs. 4, 5, graphs 4–6, show examples of the type of biological data obtained with this technique.

These two techniques cover the procedure necessary for testing most species of insects, slight modification only being necessary for individual cases. It seems, however, likely that some modifications may produce greater uniformity of results, particularly any that help to standardize the conditions after treatment. Some further experiments have already been made with this end in view.

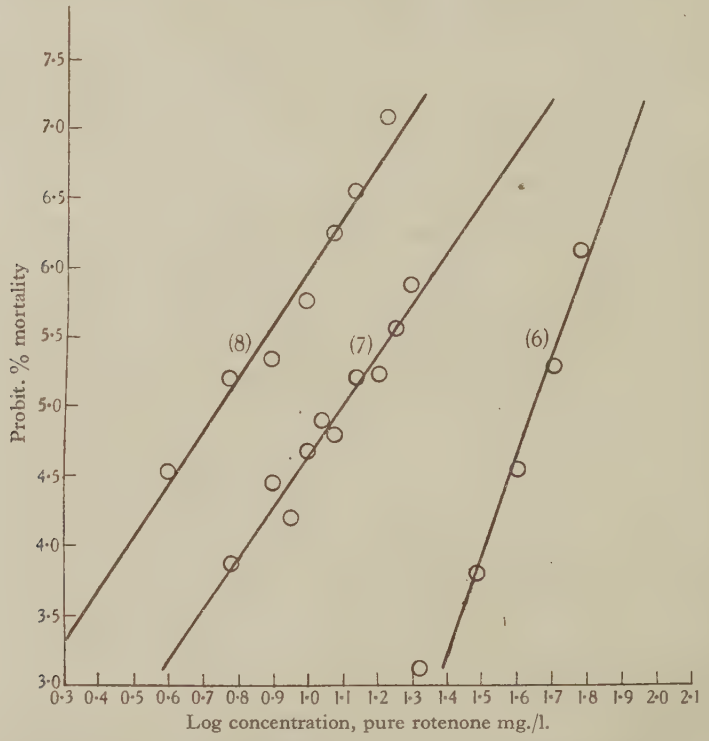
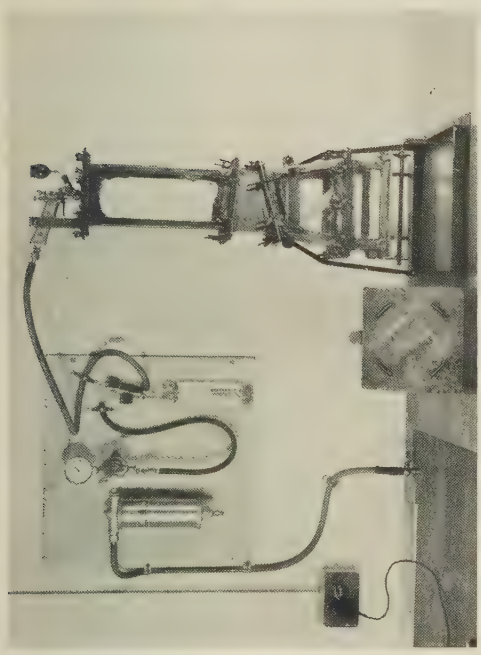


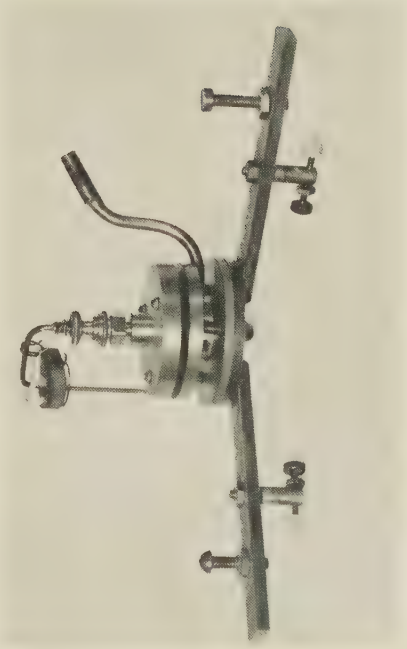
Fig. 5. Graphs nos. 6–8 showing type of data obtained with the technique described, and the resistance levels of three test insects, *Macrosiphoniella sanborni*, *Aphis rumicis* and *Oryzaephilus surinamensis*. Curves obtained using pure rotenone as toxic agent. (6) *Oryzaephilus surinamensis*; (7) *Aphis rumicis*; *Macrosiphoniella sanborni*. (See Tables 19–21.)

Summary

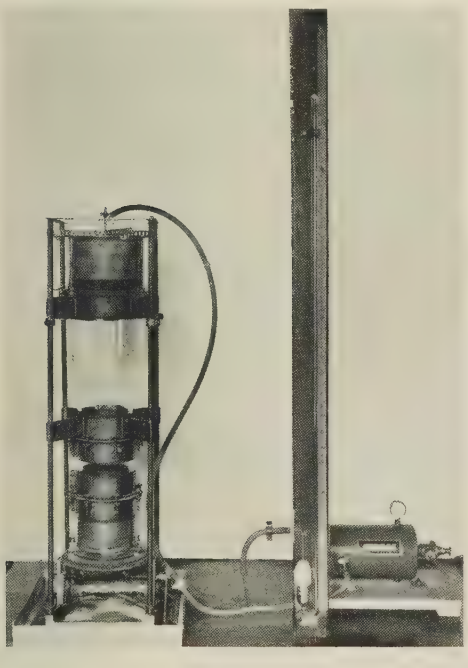
An outline of the general problem of the evaluation of liquid contact insecticides is given. A summary of the laboratory methods of evaluation already described and the reasons for the adoption of the procedure described are outlined. An account is made of the development of the spraying apparatus together with experiments on factors likely to cause variation in the replication and distribution of the deposit. The apparatus finally adopted is described and data are presented on its physical performance. The selection and rearing of test insects is described. An account is made of the factors in the technique which may cause errors in



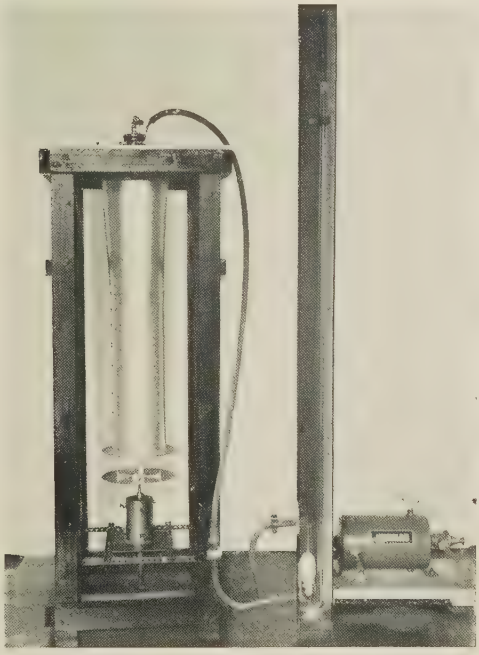
(*a*)



(*d*)



(*b*)



(*c*)

POTTER—A Laboratory Spraying Apparatus and Technique for Investigating the Action of Contact Insecticides (pp. 142–69)

the determination of the dosage-mortality data, with some experiments. The technique at present adopted is described, with examples of the dosage-mortality curves obtained.

I wish to express my thanks to Dr Tattersfield for his helpful advice and criticism and to Dr J. T. Martin for his help in the work and for various suggestions. I also wish to express my thanks to Prof. J. W. Munro of the Imperial College of Science and Technology in whose department and under whose direction the preliminary work was carried out.

I am indebted to the Hawthorndale laboratories of Imperial Chemical Industries for stocks of *Macrosiphoniella sanborni* Gill. and *Plutella maculipennis* Curtis and to the Ministry of Agriculture and Fisheries and the Colonial Development Fund for grants which have made this work possible.

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EXPLANATION OF PLATE 8*a–d*

(*a*) Original apparatus with mixing tower and settling chamber. The spray plate which fits at the bottom when in use is shown on the left of the apparatus. The air line with filter, reducing valve, pressure gauge and air-flow meter is shown on the panel.

(*b*) Photograph of apparatus used for a considerable amount of experimental work using a glass tower, scent-spray nozzle and spray table without universal adjusting device.

(*c*) Photograph of apparatus finally decided upon with metal tower, specially designed nozzle and spray table with universal adjustment.

(*d*) Photograph of atomizing nozzle and mounting.

(*Received* 25 *October* 1940)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

GENERAL MEETING of the Association held at 12 noon on Friday, 6 December 1940, in the Congregational Hall, Harpenden; the President, Mr C. T. Gimingham, in the Chair. A discussion on 'The Function of Applied Biology in War Time' was opened by Sir E. JOHN RUSSELL, F.R.S.

THE FUNCTION OF APPLIED BIOLOGY IN WAR TIME

BY SIR E. JOHN RUSSELL, F.R.S.

THE function of applied biology in war time is the same as that of any other applied science: it is to contribute as much as possible to the solution of problems of national importance. The work falls into three divisions: advisory, based mainly on existing knowledge and experience; *ad hoc* research to find new and better methods of solving particular problems; and fundamental research, to gain new knowledge, which may have no immediate practical application and yet is an essential condition of progress and in the fullness of time will afford a sound basis for further improvements in advisory and *ad hoc* work. The distinctions are gradually getting merged: pure science is becoming more and more technical in its methods and outlook, and advisory officers are constantly confronted with problems that demand some investigation before sound advice can be given. There is still need, however, for closer co-operation and interchange between advisory and research officers: the advisory officers would gain by the new information the research people could offer, and the research staff would gain from the wide experience that advisory work affords.

The most useful approach therefore to our discussion is to set out the main groups of problems likely to arise and to see how best to deal with them.

There are four general groups of problems to which applied biology could usefully contribute: (1) direct participation in the war, the so-called biological warfare; (2) maintenance of public health during the war; (3) protection of food supplies and other materials, e.g. wood, flax, etc., both in the field and in the store against deterioration by biological agents; (4) increase in the amount of food supplies.

We shall not now spend time over biological warfare. So far as is known it is not being practised by the Germans, presumably because they do not think it very practicable. If this really is so there is no point in discussing it; if it is feasible, discussion would be indiscreet. The maintenance of public health is primarily the concern of the medical authorities, but in one group of problems applied biologists could give very useful assistance: the adequate cleansing of shelters, and of persons and property of evacuated people. The first evacuation of children and mothers from London distributed enormous numbers of lice and other pests over the countryside. There were outbreaks of scabies among children who had previously known only clean conditions of life, and quantities of bedding had to be destroyed after a few nights because no other way was indicated for preventing the spread of the undesirable insects. These dreadful experiences burnt themselves into the memories of householders in the reception areas, and harassed as they were with other troubles, they were strongly disinclined to incur further risks. It was extremely unfortunate that lack of supervision should have brought this wholly unnecessary complication into the billeting problem. The aid of the biologist should certainly be sought in finding insecticides for the adequate cleansing of infected persons and materials. Pyrethrum, a valuable insecticide, suffers from the disadvantage that it induces dermatitis in certain people. J. T. Martin happens to be susceptible and offered himself for experiment; this was done in association with a medical man, Dr Hester. It was found that the dermatitis was not due to the insecticidal substances but to an oil which, it is hoped, may be removable without seriously affecting the insecticidal value. It is, however, chiefly in connexion with food supplies that applied biologists are usually concerned, and by a convention which seems to have been generally accepted they leave food production mainly to agriculturists while they devote themselves mainly to food protection. The peace-time British dietary is on the average both varied and attractive: it totals about 900–1000 lb. per head per annum (omitting milk and beverages), including about 200 lb. bread and flour, 200 lb. potatoes, 200 lb. meat, fish, poultry, etc., 100 lb. sugar, 100 lb. fruit, 100 lb. vegetables

(but this cannot be precisely estimated because of the unknown amounts obtained from private gardens), and smaller quantities of butter, cheese, eggs, etc. It is based on peace conditions, and requires easy and rapid communication with almost all occupied parts of the world because these quantities of foods could not possibly all be produced at home: in point of fact only about 40% was so produced. It is impracticable in war time and we could not hope to keep it up. The German dietary, on the other hand, is much better for war conditions: the total amount is about the same but it includes 400 lb. potatoes (i.e. 200 lb. more than we have), 220 lb. of bread, but much less meat, fruit, sugar and butter: a much larger proportion can be produced at home. The dietary is certainly physiologically adequate even though it does not make for the slim figure required by English standards of taste. In war time we have no option but to modify our dietary in the German direction: to consume less meat, fruit, sugar and butter, but more bread and considerably more potatoes. This dietary can be largely produced at home. Already even in our transition state we are producing 60% of our total food. In order to soften the fall we are encouraged to grow more vegetables in our gardens and allotments, and if we like, more soft fruit: but against the curtailment of meat, sugar and butter the ordinary citizen can do little or nothing.

This enforced change in our dietary involves a much greater production of grain and of potatoes and necessitates important changes in our agriculture including the ploughing up of grassland, and this has brought in its train a crop of problems for the applied biologist. Grassland contains a mixed population of insects, fungi and bacteria, some of which are harmful to certain plants, and though the grass grows vigorously enough to flourish in spite of damage done, other crops are not so fortunate. Chief of the pests are the wireworms which caused considerable damage during the last war and have indeed already done a fair amount of damage this year. Considerable areas of cereals had to be sown twice, involving much waste of precious seed, and yields on badly infested land suffered greatly. No good estimate of the amount of damage can, however, be made. In the 20 years peace interval something has been done on the subject and search has been made for methods of controlling wireworms. Unfortunately, the work was not on a scale commensurate with the difficulties of the subject, but it was not a peace-time problem and so progress was very limited. We are still without a cheap, effective agent for killing wireworms in the soil and still have to rely on evading it by cultivation devices. Some of these are very old, but they, along with others, are being tested by H. W. Miles at the Warburton experimental farm in Cheshire. We may hope that the investigations of Prof. James Gray of Cambridge will throw some new light on the story of the wireworms.

Another modification of agricultural practice demanded by the war is the more frequent growth of cereals, which may mean a shortening of the interval between one wheat crop and the next. This may easily lead to a spread of *Ophiobolus graminis*, the fungus which causes take-all in wheat, and to which lightish somewhat alkaline soils are very prone. This fungus occurs in grassland, and may therefore attack wheat grown on newly broken land. It is being studied in detail by S. D. Garrett at Rothamsted. Another wheat pest is *Cercosporella herpotrichoides* causing eye spot lodging; it is encouraged by the nitrogenous manuring which wheat farmers are now urged to practice. It is being studied by Mary D. Glynne.

The other vitally important crop, the potato, is interesting to the pathologist because of its liability to a large number of different diseases: happily in English practice those which might cause serious trouble are in the main controllable. *Phytophthora* was by far the worst till spraying became common, and it may indeed become even less menacing if Dr Salaman's resistant varieties can be developed. Wart disease also might have been disastrous but for the existence of resistant varieties first reported by G. C. Gough. The Colorado beetle happily does not thrive here, though of course constant watch has to be kept against it. Wireworms are, however, still troublesome, especially after old grass. The most serious losses now are those due to virus diseases which get into the seed and so attack the young plant: nothing can be done once infected seed has been sown. F. C. Bawden, Kenneth Smith and others are studying this problem; meanwhile the agriculturist tries to get round it by using seed free from virus obtained from regions too wet and windy for the aphide vectors to spread. Much still remains to be done in ensuring larger supplies of virus-free seed.

Another set of problems arises from the need to produce more fodder crops to take the place of some of the grass that has been ploughed up. During the next season (1941–2) our animals will lose not only 4 million acres of grass but a considerable amount of imported foodstuffs also, and unless we can somehow make up the deficit the animal population must be reduced, much to the detriment of post-war agriculture. Norman Wright estimates that the conversion of 2 million acres of grassland into arable in 1938–9 involved a net loss of 50,000 tons of protein equivalent. This of course is only a small fraction

of our total consumption, which he puts at 3·7 million tons per annum, but 1 million tons—27%—of this had to be imported, and these imports are now very heavily curtailed.

Fortunately, some of the fodder crops supply considerable quantities of protein equivalent. Beans are among the best of these, but they are liable to suffer badly from aphis attack especially when, as often happens, they have to be sown in spring. Control measures suitable for application on a farm scale are urgently needed. Other fodder crops readily convert ammonium salts into protein equivalent: one of the most effective is marrow-stem kale, which out of 1 cwt. sulphate of ammonia containing 23 lb. nitrogen can produce 44 lb. of protein equivalent as well as 300 lb. starch equivalent. Unfortunately kale, like some other good fodder crops, is susceptible to the attack of the turnip flea beetle, and though effective remedies for this pest have been discovered the expense of applying them tends to prevent their use on a really large scale and further work is desirable. The flea beetle is seasonal in its incidence, but when it comes in quantity it is very destructive. On our own farm we have had to sow kale twice and both sowings have failed, involving loss of seed and of time, and in the end the substitution of some other crop nothing like so good.

The advisory officers will continue to spend much of their time on a host of other pests and diseases none of which need be selected for special mention except leatherjackets, which on occasions cause much loss but can now be satisfactorily controlled. The control of fruit and market garden pests is being facilitated by the work of F. Tattersfield and his colleagues at Rothamsted on the vegetable insecticides, and of Hubert Martin at Long Ashton on insecticides and fungicides. Among administrative problems urgently needing solution is some way of examining, standardizing and controlling proprietary insecticides and fungicides.

At present considerable areas of woodland are being cut down. Some will be replanted and it is hoped that the new woodlands will be adequately guarded against pests.

The diseases and pests of animals properly come under the control of the veterinary experts, but there is a border-land where the entomologist can give valuable aid. R. P. Hobson in North Wales has investigated the sheep blow-fly, work that links up with that of the Australian workers including A. J. Nicholson at Canberra. J. Macleod in Scotland has studied the tick *Ixodes ricinus*, which causes louping in sheep. Other problems in animal disease urgently await solution.

Important developments in applied biology have led to considerable improvements in the cleanliness of milk with great advantage both to the farmer and the public. The National Institute for Research in Dairying at Shinfield, near Reading, has carried out the scientific work, and farmers, especially the younger ones, have taken it up with keen interest. It is now quite common for a young farmer to discuss with the expert the bacterial counts in samples of milk taken on this farm, and to try and explain and of course stop some undesirable increase. This has not only made milk much safer, it has also reduced the losses that used to be fairly common and which of course could not possibly be spared now. The young farmer takes kindly to microbiology.

A series of problems is associated with the so-called physiological diseases of plants. At the moment there is a tendency to put them all down to deficiencies of various sorts: boron, manganese, zinc, copper, etc. In some cases the attribution is doubtless correct, but not in all. Spectrographic methods are now available for the rapid analysis of plant tissues and the estimation of the metallic constituents; we have set up the apparatus at Rothamsted and already examined a number of affected plants: it has happened, however, that leaves supposed to be potash deficient were rich in potash, and that others supposed to be manganese deficient were really very low in potash. Obviously a good deal more work has to be done on diagnostic symptoms, though very considerable progress has already been made by W. A. Roach who is studying this subject at East Malling.

It is impossible to say how much we lose from plant diseases in the course of the year. The value of farm crops sold off the farm was before the war about £34 millions: in addition, fruit and vegetables exceeded £22 millions in value, and there was some £8 million worth of glasshouse produce and nursery stuff: in all some £64 millions, to say nothing of what was consumed by the animals on the farm. Any estimate of loss can be only a guess, but those who have had the courage to attempt it do not put the loss at less than 10%, which would make it of the order of £10 million or more per annum. Naturally we can never hope to do away with this entirely, but it should be capable of considerable reduction.

The storage of food gives rise to many biological problems. During the last war great losses of grain arose from the depredations of weevils, and, in places, of mice also. The weevils can now be controlled thanks to the pioneering work of Lefroy and the modern critical work of J. W. Munro, who has also dealt ably with other problems of food storage. The storage of friut is complicated by the activities

of moths, but these can be dealt with. The subject of food storage still presents difficult problems, but happily the position so far as scientific assistance is concerned is much better than it was in the last war.

The war-time activities of applied biologists would be greatly enriched if they could plan their work so that it could be subjected to statistical examination. It is particularly easy to go wrong about field observations: the first one, if it is striking enough, is apt to form the basis of a generalization, and if the first three or four happen to be more or less in agreement with it the generalization is held as amply proved. The ability to make really objective impartial observations is rare, and to get anything out of them is rarer still. Fortunately, in recent years statistical methods have been applied in biological research, notably by R. A. Fisher, J. B. S. Haldane and others. Still more recently statisticians, particularly F. Yates, have worked out sampling methods by which field observations can be put on to a proper systematic basis, and completely objective tests can be applied to see how far one set of observations is related to another. A striking example of the value of statistical methods is given by C. B. Williams's studies of insect populations as sampled by the light trap: he established close relations between the number of certain insects and the weather conditions of specified preceding periods. An important feature of the new methods is that they allow of an estimate of the error of the relationship, so that one knows what degree of trustworthiness attaches to it. Another well-known example is the work on precision records of wheat growth carried out at various centres, which has abundantly demonstrated the value of well-planned systematic observations. The war is affording many opportunities for work of this kind: a considerable number of surveys of different sorts will be made, and it is very little more trouble to do them on a proper plan so that they yield information of value, than to do them in a mere haphazard way where the results are far less satisfactory.

Although food production is by convention regarded as the province of the agriculturist there are several directions in which mycologists and entomologists can directly play a part. During the last war it was reported that the Germans had found a fungus which grew on an artificial medium of simple salts and carbohydrate and out of these substances synthesized protein and fat suitable for human consumption. Samples were obtained and an investigation was made by A. Chaston Chapman who gave the account in his Presidential Address to the Royal Microscopical Society in 1926.[1] Unfortunately, with the coming of peace the subject was dropped, but presumably the cultures still exist. So far as I know the only use made of yeast in peace time for direct food production is in the manufacture of Marmite.

I am not prepared to offer an opinion as to how far it will be necessary to enlist micro-organisms in the production of human food, but there is a very good case for investigation into their use for producing animal food. The ploughing up of grassland will, as already stated, reduce the quantity of protein equivalent available to animals unless special care is taken, and the activity of yeasts and other fungi offers possibilities that should be explored. Fortunately there is no shortage of ammonium salts, and certain other simple nitrogen compounds could no doubt be made available in large quantities. To some extent it may be possible to feed these direct to animals and so make up for part of the missing protein. But the amount thus given can only be small. On the other hand, yeasts and many fungi can convert these simple nitrogen compounds into protein, which would probably be of better nutritive value to the animal than the original compounds.

The supply of carbohydrate for the organisms would present difficulties. The most self-contained scheme, of course, would be to grow algae in a culture solution, if an alga could be found that animals could eat with advantage. Otherwise some carbohydrate must be found. Sugar-beet factories have certain sugar by-products and wastes and might be able to add this protein industry to their own activity. Possibly, however, straw could be utilized. At present farmers produce enormous quantities of straw which are not fully utilized. The classical solution of the problem was to feed the oat straw and some of the wheat straw, but to convert the rest, including the barley straw, into farmyard manure. So long as the old method of winter feeding of cattle in yards was practicable the method worked well, but nowadays it is not. Hutchinson & Richards at Rothamsted worked out a process for converting

[1] A. Chaston Chapman, *J.R. micr. Soc.* 1926, pp. 1–17. The carbohydrate can come from the hydrolysis of cellulose, e.g. wood or paper. Chaston Chapman quotes from a lecture by Hayduck in Berlin in 1916: 'When we can convert our evening papers into sugar so rapidly that we are able the following morning to eat for breakfast the albumen prepared from them, then indeed shall we have solved one of the great problems of the century.'

The biochemistry of fat production by fungi was studied at the Lister Institute by Dr Ida Smedley Maclean during the period 1919–26.

the straw into manure by micro-organisms in a compost heap instead of in the cattle yards, and this has proved very useful in garden and nursery practice, though it is often difficult, i.e. expensive on farms.

Chemists have devised a method for converting straw into fodder for the livestock, but it involves chemical processes which farmers may be reluctant to carry out by themselves. The straw could of course be carted to a factory for treatment, but this is costly and tedious, and would greatly restrict the possibilities of the method. Possibly the straw could be made into paper with protein production as a by-product, but this also involves much cartage. But while farmers could probably make little headway with a chemical process they would have less difficulty with a biological process; as we have already seen our farmers take kindly to micro-organisms. In eastern Europe it is quite common to find a distillery attached to a farm where alcohol can be produced from grain and potatoes. If it were possible to devise some method whereby straw could be used as the source of carbohydrates for micro-organisms engaged on protein synthesis something useful might result.

About another food production activity there is fortunately no uncertainty. Bees are commonly regarded as very minor agents in food production: actually they are not. Dr Butler tells me that the value of the stocks in England and Wales probably exceeds £2 million, and the annual production of honey is about 120,000 cwt., worth about £1 million. The number of colonies is probably 260,000, and the number of bee-keepers about 65,000. But there is room for many more: happily interest is increasing. The numerous problems associated with bees in health and disease are now well in hand: Dr Butler has been given staff and facilities and we may confidently expect a series of important results from him. Most people think of bees simply as honey producers: they are probably even more important as pollinators of fruit and of certain farm crops.

Finally, a product of fungal activity which is not a food but a drug is of considerable importance. Ergot has hitherto come to us mainly from Spain but also from Portugal and Poland, in all of which countries the rye crop suffers from attack by ergot, and as a kind of by-product the growers are able to sell the ergotized rye from which the alkaloid is extracted. It is a muscle stimulant, used in midwifery practice, and our total requirements are considerable. With the partial failure of the Spanish crop the usual supply is cut off and we shall probably remain short during the war. It should not be difficult, however, for an agriculturist and a mycologist jointly to supervise the growth of ergotized rye in this or some accessible country so as to give us the necessary supplies. We already have the disease in this country, so there is no case of introducing anything new, and as its rate of spread is very limited it constitutes but little danger for wheat, oats and barley, though it does attack grass. Békésy has studied methods of doing this in practice.[1]

One could of course multiply problems, but these are the urgent ones as I see them, and it is the business of applied biology to get down to them. The position is in many ways easier than in the last war. The Research and Advisory Services are much better organized: the staffs know each other and the extent to which they can collaborate. There are many more applied biologists now than there were in 1914, and, further, their services will be retained. It is true that our Association was in existence in the last war and had indeed been going for 10 years, but it had only just started the issue of its own Journal, and few meetings were held during the war. The Association is now in a much stronger position and its members have already shown their ability to deal with biological problems. The main problem now is to make the best use of our very good resources.

In the last war the organization was centralized in association with the Food Production Department. In this war the advisory and executive activities are on a regional basis while the administrative and the research services are centralized. The regional basis has the advantage that it appeals better to farmers and is less likely to disturbance by enemy action than centralized organization. But the present division has the disadvantage that the various groups may not be sufficiently in touch with each other: in particular, the research service and the advisory service should have the closest possible contacts. During war time a great deal of improvization has to be done, but this presents no insuperable difficulties so long as the problem is sufficiently clearly stated. We hope as a result of to-day's meeting that the weaknesses of the present arrangement may be fully set out in order that they may be rectified and that suggestions for improvement may emerge which will enable us to deal with the numerous and varying problems presented by the war. Flexibility is essential.

Finally, when peace comes we must ensure that war-time problems are not immediately shelved, for all history shows the fallacy of assuming that wars will cease.

[1] Békésy, N. V. Über parasitische Mutterkornkulturversuche, *Zbl. Bakt.* Abt. 2, 1938, **99**, 321–32.

Prof. J. W. Munro, instancing how old methods of testing scabies were still in use, pointed out how the country had failed to anticipate the problems that were bound to arise in war time. Food-storage research had been recommended 20 years ago, but it was only in the last few years that research on the deterioration of stored grain had been taken up actively. He emphasized the need for providing additional facilities for testing the effects of insecticides on foodstuffs both for human and for animal consumption, particularly with regard to the tainting and poisoning of these and the rendering of them unpalatable. He referred to the conditions arising in air-raid shelters and pointed out a problem of immediate importance, namely, the extent to which useful insecticides like pyrethrum could be used in the presence of hypochlorite, which was used as a general disinfectant but particularly against spread of the common cold. He pointed out the need for planning for post-war development and suggested the formation of a reconstruction committee in applied biology.

Later, Prof. Munro referred to the important work on rats and mice being undertaken by the Bureau of Animal Population in Oxford, and expressed surprise that it had not received mention. He referred briefly to the Bragg Committee on Scientific Food Policy and to the Hankey Committee as two bodies directly concerned with the employment of scientists and with the application of scientific knowledge to present-day problems. He referred to the Standing Conferences on Pest Infestation of Produce and on Rodent Control, given formal constitution by the Department of Scientific and Industrial Research and by the Agricultural Research Council respectively, and emphasized how this new development made for enhanced co-operation with all branches of industry and had already greatly simplified the application of scientific work to industrial and agricultural problems.

He suggested that the applied biologist should not be content with the publication of his scientific work but should follow it up until his results were used in practice in the field.

Mr J. C. F. Fryer said that as regards agricultural entomology, the war had as yet thrown up no new problems for the biological investigator to solve but had merely altered the emphasis—the wireworm problem, for instance, being of much greater importance now when grassland was being converted to arable than when the reverse procedure was taking place. Whether the position would remain the same as the war proceeded it was difficult to foresee: supplies of the different insecticides and fungicides, for instance, which at present were reasonably adequate, might begin to run short and in that event it would be necessary, before the shortage became acute, to devote much attention to the development of substitutes. In the meantime, however, the urgent need was to secure the application in practice of the great volume of biological knowledge already existing. As an instance of such application, he mentioned the wireworm sampling work now being undertaken by the Ministry of Agriculture and Fisheries and the Advisory Entomologists, with the object of reducing the area of heavily infested land to be sown with highly susceptible crops. Furthermore, it was hoped to reduce greatly the losses from pests which could be controlled by proved measures; thus, leatherjackets had been responsible for great damage which could almost wholly have been prevented by the use of poisoned bait; and similarly, the loss of kale and other *Brassica* crops which was so much in evidence last summer need not have occurred if spraying or dusting had been carried out in time. In order to meet these various needs, the Advisory Entomological Service had already been strengthened by the addition of twenty-eight entomologists or other biologists whose names were on the Central Register and some twenty-nine others. Possibly still further expansion would be needed, but in this connexion there was one difficulty that should not be underestimated. Practical work among farmers required at least some knowledge of agriculture and the farmers' technique, and experience had shown that it was not all entomologists or biologists who had the necessary qualifications for the work. He also supported Prof. Munro's suggestion that consideration should be given now to the formulation of programmes of work that should be pursued in peace time so that the activities in biological science started under the stimulus of war should not, as on the previous occasion, be dropped immediately on the return of peace.

Mr G. E. Blackman stated that many biologists who were not directly concerned with agriculture had high hopes when the Central Register was instituted that they would be usefully employed on war-time problems. But after fifteen months, when the official attitude seemed to be that biologists were not wanted, many had a sense of frustration. They saw that in several of the Ministries there were problems to solve but no one to solve them. This appeared to be due to the lack of personnel with the proper biological outlook and knowledge to appreciate that the problems were there and were capable of solution. Just as the Air Ministry had a Department to deal with suggestions and inventions so there should be instituted a *Technical Biological Committee* to act in a similar capacity. Furthermore,

the Committee should have more than advisory status. They should be able to initiate research and also to ensure that there was proper co-ordination and co-operation between the various research workers and research institutions.

Mr F. C. Bawden said that the ultimate aim of applied biologists working in agriculture in both peace and war was to improve methods of food production, but before the war the economic system rarely encouraged them to pursue this aim wholeheartedly. Discoveries promising increased yields were mixed blessings, for bumper crops too often brought ruin to the farmer, and the agricultural policy was one of artificial price control based on crop restrictions. In war time, on the other hand, maximum production of food at home became essential, and the immediate function of applied biologists was to see that all knowledge likely to assist in this was fully used. He would like to support Mr Blackman's suggestion that an advisory committee of applied biologists be formed, for this should be able to lead the directors of policy through the mental revolution needed to change from a policy of crop restriction to one of increased production. In many subjects other than agriculture, such a committee would be useful in foreseeing biological problems that might arise during the war and in helping the administrators to plan ahead so that these might be avoided, instead of, as now too often happened, as, for example, with air-raid shelters, waiting for the problems to become acute before tackling them.

Sir John Russell had shown us that the potato produces the greatest yield of food per acre, and that in Germany the consumption of potatoes was twice that in this country. More potatoes should be grown here, but before this was done adequate supplies of healthy seed must be ensured and means devised for the consumption of the crop. There was little point in ploughing up grassland merely to plant potato seed incapable of giving large yields, or in producing larger crops if the public would not eat them. Our knowledge of manuring, cultivation and pest control was considerably ahead of practice, and yields on many farms were woefully low. Increased food production might result more easily if more attention were given to yield per acre instead of concentrating on an unrestricted plough-up policy, for it was unlikely that a man farming 50 arable acres badly would farm 75 well. Epidemics of cereal or potato diseases would now be a calamity. Control methods were known for most, and the necessary knowledge and machinery to prevent such epidemics should be available generally. In peace time the help applied biology could give to agriculture and industry was often greatly hampered by political and commercial interests. A committee of strong-minded biologists might do something to ensure that the same thing did not happen now.

Dr R. C. Fisher, referring to the prevention of deterioration of the national stocks of timber through insect attack, drew attention to the need for closer co-operation with administrative officials. As in agricultural entomology, no new timber insect problem had yet arisen in this country as a result of the present war, but use was not being made of information upon methods of prevention and control available from the results of research on timber insect problems that arose during and after the war of 1914–18. No steps had been taken to prevent the recurrence of these problems: on the contrary, the view was taken by those responsible for the custody of the nation's timber resources that other problems were of greater importance, and that the question of dealing with insect attack need not be considered until it arose—in other words, until after the damage had been caused. He emphasized the importance of bringing to the notice of the authorities concerned the value of regular inspections of stocks of timber, and the application of simple preventive measures as means of avoiding serious infestation.

Dr H. W. Miles said that the aspect of applied biology with which he was concerned was the work of the advisory biologist in agriculture. In normal times farmers had been encouraged to make their first appeal for advice on the control of pests and diseases in farm crops to the county agricultural staff. The members of the county staff were able to deal with enquiries concerning common pests and established control measures but passed on to the specialist adviser those enquiries which were too complex to be dealt with as a matter of routine. With these more difficult enquiries the specialist advised or made recommendations to the county staff who in turn passed the information back to the farmer and co-operated with him in carrying out the suggestions. In this way the adviser only made contact with the farmer through the county agricultural service and might not know whether his advice was adopted.

Under war conditions the county staff was preoccupied with administration and had little time available for work on plant protection. Farmers therefore appealed directly to the provincial advisory

officer who was thus called upon to deal with routine enquiries on pest control, enquiries concerning the pests of newly ploughed grassland and enquiries from grassland farmers concerning the pests of arable crops with which quite often they had little or no experience. The provincial adviser, single-handed, was quite unable to satisfy all these demands, and a considerable expansion of the advisory service was a war-time necessity.

The direct contact between farmer and specialist adviser brought about by the war-time needs of agriculture was in many ways desirable, and was likely to have far-reaching results in the development of crop protection in this country. The advisory officer would have to express his instructions in simple, direct and practical terms and be able to demonstrate pest control measures in the field. The farmer might become more open-minded and willing to adopt new measures and equip himself with spraying tackle to carry out measures of pest and disease control. At present scarcely one farmer in a hundred had even the simplest of spraying or dusting machines and could not apply insecticides or fungicides to his crops. The contact between farmer and specialist adviser should ensure that the farmer gets the advice which meets his special needs and that the adviser sees his recommendations carried out in the right way at the correct time and with the type of equipment most likely to give a satisfactory measure of crop protection.

Mr W. P. K. Findlay drew attention to the need for biological advice in Departments which at first sight might be thought to have no biological problems. The need for assistance from applied biologists was obvious in dealing with pests and diseases of agricultural crops, but was it sufficiently realized that the prevention of deterioration in all organic materials in storage was essentially a problem in applied biology? This question affected many different interests which came under the aegis of various Departments which perhaps did not always realize that a solution to their problems might already be known. He instanced the serious wastage in sandbags which had been allowed to occur because preservative treatment had not been applied, although methods of preservation were already known. One of the major problems was to get the advice already available to those who were confronted with problems which required immediate solution.

Mr S. D. Garrett suggested that the responsibility for anticipating biological problems likely to arise in time of war lay with applied biologists themselves, as they were in the best position to foresee the advent of such problems; their responsibility was therefore not discharged until they had made every effort to stir the administrators into appropriate action. The value of applied biologists to general agriculture was to be judged by the extent to which they influenced general farming policy, rather than by the production of spectacular cures for diseases and pests, the value of which was generally limited by their expense, and by the fact that disease and pest control was only one of many problems to be solved simultaneously by the farmer. Applied biologists ought therefore to be more frequently consulted during the planning of general agricultural policy.

Dr W. A. Millard, speaking in respect of agriculture only, said that it was strange that no one had mentioned the War Agricultural Executive Committees during the discussion. In Yorkshire, the three War Agricultural Executive Committees took over amongst them almost the whole of our agricultural staff, numbering fifteen, to act as Advisory, Liaison or Executive Officers, and to this number they subsequently added seven more, most of whom were old colleagues or old students. We, therefore, remained in close touch with them, and they made constant demands on our services. Not only were our Ministry Advisers in soil chemistry, entomology and mycology fully occupied, but all the staff were taking an active part in advisory and other war work. For example, the chemists were exploring the possibility of waste products as fertilizers with much success, and the feeding value of silage, etc.; the veterinary staff were in constant demand, and the botanical and zoological staffs were helping the Ministry Advisers in mycology and entomology. We have no reason whatever to complain that our services were not being fully utilized. If this obtains in so large a county as Yorkshire, why should it not do so in other counties where there is some central Agricultural Department or Institute? Co-operation between the War Agricultural Executive Committee and the Agricultural Department had no doubt been easy in Yorkshire, since our county work before the war was centralized in the Department, but this should not be difficult to achieve even where County and Department were previously separate units.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1940

THE Officers and Council of the Association were as follows:

President: C. T. GIMINGHAM, B.Sc., F.I.C.

Vice-Presidents: G. E. BLACKMAN, M.A., F. R. PETHERBRIDGE, M.A.

Hon. Treasurer: W. C. MOORE, M.A.

Hon. Secretaries: General and Botanical: W. P. K. FINDLAY, M.Sc.; Zoological: H. F. BARNES, M.A., Ph.D.

Hon. Editors of Annals of Applied Biology: General and Botanical: Prof. W. B. BRIERLEY, D.Sc.; Zoological: C. T. GIMINGHAM, B.Sc., F.I.C.

Council: *G. C. AINSWORTH, Ph.D., G. E. BLACKMAN, M.A., *Miss W. E. BRENCHLEY, D.Sc., S. D. GARRETT, M.A., *R. V. HARRIS, B.Sc., H. G. H. KEARNS, Ph.D., *O. B. LEAN, B.Sc., H. C. F. NEWTON, Ph.D., F. R. PETHERBRIDGE, M.A., A. ROEBUCK, N.D.A., *W. R. THOMPSON, D.Sc., F.R.S., *W. M. WARE, D.Sc. * New members for 1940.

At the well-attended Annual General Meeting held in the rooms of the Linnean Society, London, on Friday, 9 February 1940, the view was generally expressed that the Association should, so far as possible, carry on its normal activities during the war. A second meeting held in London on 15 March was also well attended, and at a meeting held in Harpenden on 6 December almost a record number of Members was present, so it is evident that the meetings of the Association provided a much appreciated occasion for Members and their friends to meet and discuss problems of the day. The attendance at meetings was on the average fifty Members and twelve visitors, which is the highest for several years—a remarkable proof of the enthusiasm of Members in these days of difficult travelling.

It was with regret that the Association had to record the deaths of the following Members, all of whom joined the Association over 20 years ago: in 1939, Prof. P. A. van der Bijl and Dr A. H. Trow; and in 1940, Mr A. J. Murphy and Mr F. A. Mason, and very recently that of Mr D. Ward Cutler who was Zoological Editor of the *Annals* from 1921 to 1932.

Twenty-six new Members were elected, nearly twice as many as in the previous year. The Association now numbers as far as can be ascertained 290 Members including twelve Honorary Members, a decrease of forty-six on the previous year. This decrease is more apparent than real for it was found that a number of Members included in the 1939 list had allowed their membership to lapse several years previously and their formal resignations have only now been received. Of the ordinary Members as far as is known 245 are resident in the British Isles and 33 in the Empire or foreign countries.

The following communications were brought before the Association during the year:

9 Feb.: Presidential Address by C. T. GIMINGHAM: 'Some recent contributions by English workers to the development of methods of insect control.'

15 Mar.: Discussion on 'Efficiency with economy in the control of plant diseases and pests'. H. MARTIN: 'The general problem and the transition to war conditions.'

M. H. MOORE spoke on problems concerning the control of plant disease, while S. G. JARY dealt with entomological problems. Questions of spraying machinery were dealt with by CORNELIUS DAVIES and the practical aspects of fruit spraying were discussed by A. J. WOOLDRIDGE.

6 Dec.: Discussion on 'The function of applied biology in war time'.

The opening address was given by Sir JOHN RUSSELL and many other members contributed to the discussion which followed. At the end of the meeting a resolution was passed asking the Council to 'bring to the notice of such authorities as they think desirable, some of the pressing problems which exist to-day in applied biology and to discuss with them the means of utilizing to the best advantage the services of applied biologists'.

On 9 February and 15 March the Association was kindly allowed the use of the Linnean Society's rooms in Burlington House and the Council wishes to take this opportunity of thanking the Linnean Society on behalf of the Association for their hospitality.

At the invitation of the British Mycological Society a joint committee was set up including representatives both of that Society and of the Association to discuss systems of recommending the use of proprietary insecticides and fungicides with a view to getting some official action taken. This Committee has now prepared a Report which has been submitted to the Minister of Agriculture and Fisheries.

At the outbreak of war it was decided to make every effort to continue publication of the *Annals of Applied Biology*, but it soon became clear that economies would be necessary in order to meet increased costs of production, a shortage of paper, and possibly a diminished income. These needs were met by changing the format of the *Annals*, the alterations being carried through in time for No. 1 of the volume for 1940. Volume 27 (1940) comprised pp. x + 576 and 22 plates, as against pp. xii + 849 and 43 plates for volume 26. Because of the changed format which allowed of a notable increase in the amount of textual matter per page, the 1940 volume contained approximately as much material as that for 1939. Including papers published as 'Proceedings', volume 27 comprised 53 papers and 21 reviews, 42 of the former being by Members of the Association. The papers may roughly be classified as follows: general applied botany, 9; mycology and fungal diseases, 7; bacterial diseases, 1; viruses and virus diseases, 5; general applied zoology, 4; entomology and insect pests, 7; plant protection, insecticides and fungicides, 15; helminthology and nematode diseases, 2; applied microbiology, 2; general, 1.

The several issues of the *Annals of Applied Biology* were published on the following dates: No. 1, 11 March; No. 2, 23 May; No. 3, 14 August; No. 4, 26 November.

REPORT OF THE HON. TREASURER FOR THE YEAR ENDING 31 DECEMBER 1940

During the year ending 31 December 1940, subscriptions and entrance fees amounted to £412. 17*s*. 0*d*., an increase of £151. 12*s*. 6*d*. over last year. This amount includes arrears of £7. 10*s*. 0*d*. not yet paid but considered good, as well as a sum of £50. 15*s*. 6*d*. previously assumed to be bad debts. In addition, the arrears of £110. 17*s*. 0*d*. regarded as good on 31 December 1939, and included among the Association's assets in last year's Balance Sheet, were collected during the year.

Income from the sale of the current volume of the *Annals of Applied Biology* and from reprints amounted to £706. 6*s*. 4*d*., a decrease of £81. 12*s*. 5*d*., due to a falling off in sales of the volume. The size of the *Annals* was 576 pages compared with 849 pages in 1939, but owing to the change in format made at the beginning of the year, there was little reduction in the amount of matter contained in the volume. The cost of printing was £1054. 10*s*. 3*d*., a reduction of £194. 9*s*. 2*d*.

The income for the year exceeded the expenditure by £110. 8*s*. 4*d*., and after all obligations have been met, the assets of the Association amount to £760. 17*s*. 4*d*. of which £515. 9*s*. 3*d*. is represented by National Savings Certificates and £183. 17*s*. 0*d*. is contributed by the estimated value of the stock of the *Annals*. The financial position of the Association is therefore satisfactory. There is, fortunately, no indication of an appreciable fall in the active membership, and provided the decline in income from current volume sales does not become more marked, it should be possible to avoid reduction in the size of the *Annals*, especially if members pay their subscriptions promptly and make every endeavour to introduce new members.

W. C. MOORE.
Hon. Treasurer

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1940 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To Estimated Value of Stock, 1 January 1940		177 16 0	By Sales—Current Volume		579 10 10
To Cambridge University Press		1054 10 3	By Sales—Back Volumes, Parts and Sets		54 13 8
			By Sales of Reprints		126 15 6
			By Contribution towards cost of Papers		12 0 0
			By Advertisements		2 0 0
			By Estimated Value of Stock, 31 December 1940		183 17 0
			By Balance, carried down		273 9 3
		£1232 6 3			£1232 6 3

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1940 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To *Annals of Applied Biology*, balance brought down		273 9 3	By Members' Subscriptions		400 15 6
To Printing and Stationery		8 15 6	By Entrance Fees		12 1 6
To Postages and Cheque Stamps		9 15 1	By Interest on National Savings Certificates		15 9 4
To Subscription—Parliamentary Science Committee		5 5 0			
To Sundry Out-of-Pocket Expenses of Editors, Secretaries and Treasurer		16 9 2			
To Audit Fee		4 4 0			
To Balance, being Excess of Income over Expenditure for the year		110 8 4			
		£428 6 4			£428 6 4

BALANCE SHEET, 31 December 1940

Liabilities and Surplus	£ s. d.	£ s. d.	Assets	£ s. d.	£ s. d.
Sundry Creditors:			Cash at Bank:		
Cambridge University Press	291 10 3		Current Account	365 9 7	
Audit Fee Reserve	4 4 0		Deposit Account	1 0 0	
Sundry Expenses	7 8 9				366 9 7
		303 3 0	Debtors for Subscriptions considered good		7 10 0
Subscriptions and Entrance Fees, paid in Advance		9 5 6	301 National Savings Certificates		515 9 3
Excess of Assets over Liabilities:			Stock of *Annals of Applied Biology* at estimated value		183 17 0
As Balance Sheet of 31 December 1939	650 9 0				
Add: Balance of Income and Expenditure Account for 1940	110 8 4				
		760 17 4			
		£1073 5 10			£1073 5 10

W. C. MOORE, *Hon. Treasurer*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association according to such books, vouchers and documents.

(Signed)
H. J. COX & CO.
Incorporated Accountants
Auditors

HARPENDEN, 28 *February* 1941